EXPRESSION OF CYCLOXYGENASE -2 IN CERVICAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL VARIABLES

Dissertation submitted in

partial fulfilment of the requirements for the degree of

M.D. (PATHOLOGY)

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MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

THE TAMIL NADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2016

CERTIFICATE

This is to certify that this Dissertation entitled **"EXPRESSION OF CYCLOXYGENASE -2 IN CERVICAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL VARIABLES**" is the bonafide original work of **Dr.DIVYA. N**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the TamilnaduDr.M.G.R Medical University to be held in April 2016.

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DECLARATION

I, **Dr.DIVYA.N,** solemnly declare that the dissertation titled "**EXPRESSION OF CYCLOXYGENASE-2 IN CERVICAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL VARIABLES**" is the bonafide work done by me at Department of Pathology, Institute of Obstetrics and Gynecology, Egmore, Chennai under the expert guidance and supervision of **Prof.Dr.GEETHA DEVADAS.M.D.D.C.P,** Professor of Pathology, Institute of Pathology, Madras Medical College. The dissertation is submitted to the Tamil Nadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr. DIVYA. N**

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INSTITUTIONAL ETHICS COMMITTEE
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CERTIFICATE OF APPROVAL

To
Dr. N.Divya,
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Dear Dr.N.Divya,

The Institutional Ethics Committee has considered your request and approved your study titled **"Expression of cycloxygenase 2 in cervical carcinoma and its correation with clinicopathological variables". No.11102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

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2.	Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
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11.	Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee
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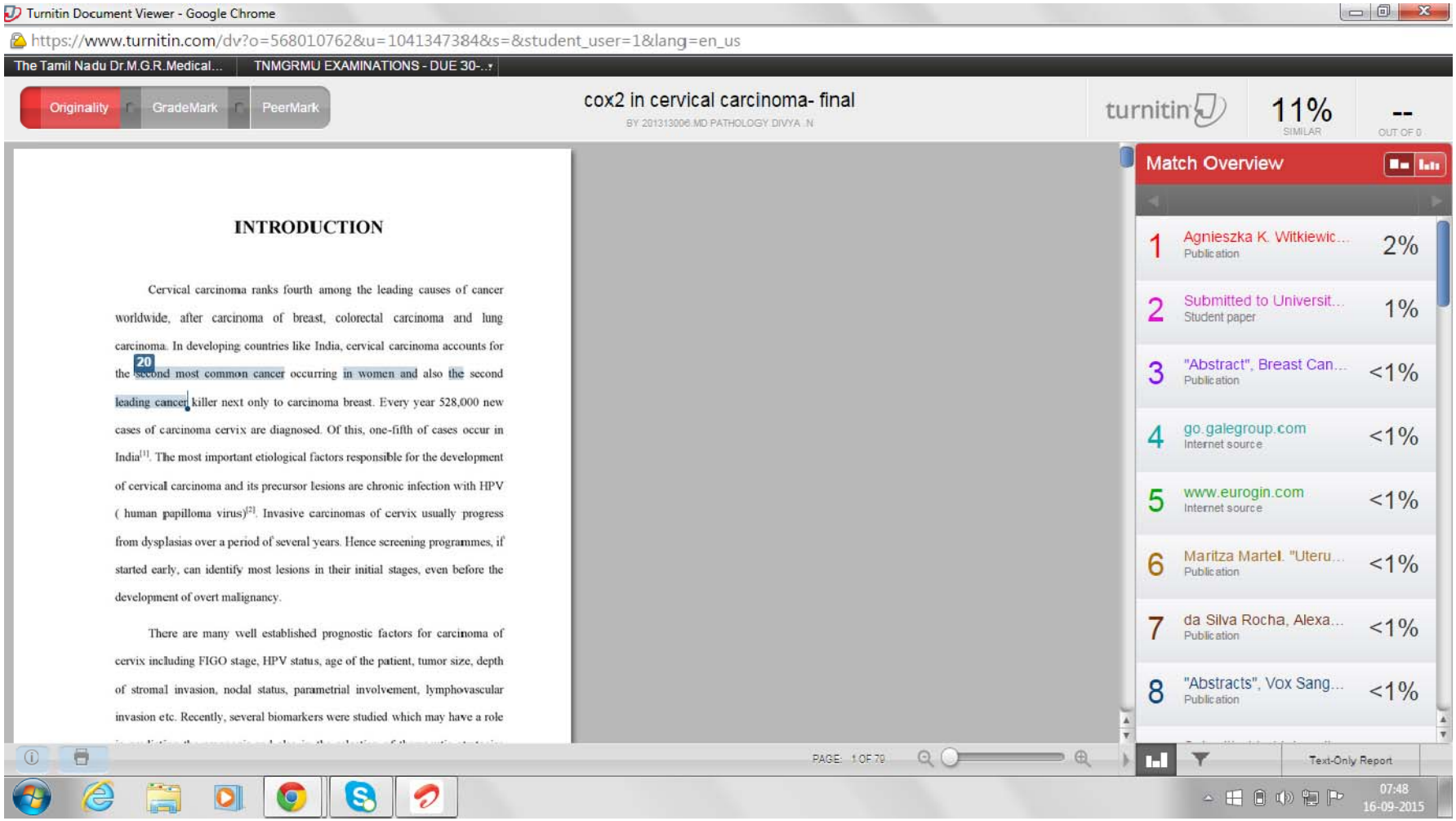
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INTRODUCTION
Cervical carcinoma ranks fourth among the leading causes of cancer worldwide, after carcinoma of breast, colorectal carcinoma and lung carcinoma. In developing countries like India, cervical carcinoma accounts for the second most common cancer occurring in women and also the second leading cancer killer next only to carcinoma breast. Every year 528,000 new cases of carcinoma cervix are diagnosed. Of this, one-fifth of cases occur in India[1]. The most important etiological factors responsible for the development of cervical carcinoma and its precursor lesions are chronic infection with HPV (human papilloma virus)[2]. Invasive carcinomas of cervix usually progress from dysplasias over a period of several years. Hence screening programmes, if started early, can identify most lesions in their initial stages, even before the development of overt malignancy.
There are many well established prognostic factors for carcinoma of cervix including FIGO stage, HPV status, age of the patient, tumor size, depth of stromal invasion, nodal status, parametrial involvement, lymphovascular invasion etc. Recently, several biomarkers were studied which may have a role
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There are many well established prognostic factors for carcinoma of cervix including FIGO stage, HPV status, age of the patient, tumor size, depth of stromal invasion, nodal status, parametrial involvement, lymphovascular invasion etc. Recently, several biomarkers were studied which may have a role in predicting the prognosis and also in the selection of therapeutic strategies that can be helpful to further improve patient survival for carcinoma of cervix. Of these one important molecular marker is cyclooxygenase 2 (COX2).

1

ABBREVIATIONS

SCC	:	Squamous cell carcinoma
ADC	:	Adenocarcinoma
EGFR	:	Epidermal growth factor receptor
WHO	:	World Health Organisation
IHC	:	Immunohistochemistry
HPV	:	Human papillomavirus
FIGO	:	International Federation of Gynecological Oncologists
COX	:	Cycloxygenase
CIN	:	Cervical Intraepithelial Neoplasia
VIA	:	Visual Inspection with Acetic acid
VILI	:	Visual Inspection with Lugol’s Iodine
LSIL	:	Low grade squamous intraepithelial lesion
HSIL	:	High grade squamous intraepithelial lesion
OCP	:	Oral Contraceptive Pills
CT	:	Computerised Tomography
MRI	:	Magnetic Resonance Imaging
VEGF	:	Vascular Endothelial Growth Factor

CONTENTS

ABSTRACT

Cervical carcinoma ranks fourth among the leading causes of cancer worldwide, after carcinoma of breast, colorectal carcinoma and lung carcinoma. In developing countries like India, cervical carcinoma accounts for the second most common cancer occurring in women and also the second leading cancer killer next only to carcinoma breast.There are many well established prognostic factors for carcinoma of cervix including FIGO stage, HPV status, age of the patient, tumor size, depth of stromal invasion, nodal status, parametrial involvement, lymphovascular invasion etc. Recently, several biomarkers were studied which may have a role in predicting the prognosis and also in the selection of therapeutic strategies that can be helpful to further improve patient survival for carcinoma of cervix. Of these one important molecular marker is cycloxygenase 2 (COX2).

In our study, we evaluate thepattern of COX-2expression in cervical carcinomas and its association with various clinicopathological variables like age of the patient, stage, menstrual status, tumor size, parametrial involvement, vaginal involvement, nodal status and histological grade of tumor.

AIMS AND OBJECTIVES

To assess the expression of Cycloxygenase 2 (COX-2) in invasive cervical carcinomas. To compare the clinical behaviour of cervical carcinomas and cyclooxygenase 2 expression. To correlate the expression of cyclooxygenase -2 (COX2) with various clinicopathological variables. To assess the value of COX2 as a prognostic and predictive marker.

MATERIALS AND METHODS

For the 50 patients included in the study,the paraffin tissue blocks were cut and stained with eosin and hematoxylin.Immunohistochemical analysis of marker

COX2 was done in all these cases and the results compared with the clinical and morphological parameters to study the efficiency of marker (COX2).

RESULTS

When the menstrual status and stage of the disease were compared, it was noted that menstruating females presented in early stages of the disease, whereas postmenopausal females presented in advanced stages of disease. This correlation was statistically found to be significant with a p value of 0.004.

In our study, it was noted that smaller tumors showed less COX2 expression whereas it was significantly higher for larger tumors. This was found to be statistically significant with a p value of 0.007.

On comparing the COX2 expression with nodal status of the patients, it was noted that among cases with enlarged nodes, 44.2% had positive COX2 expression. This was found to be statistically significant with a p value of 0.026.

On comparing COX2 expression with clinical stage of the disease, it was noted that COX2 expression was more in tumors of higher clinical stage.

CONCLUSION

In our study, expression of COX2 was found to be correlating with many of the established poor prognostic factors. Hence it can be concluded that this study is pointingtowards the possibility of considering COX2 as a poor prognostic marker for cervix carcinoma

key words: COX2, FIGO, HPV, cervix carcinoma.

INTRODUCTION

Cervical carcinoma ranks fourth among the leading causes of cancer worldwide, after carcinoma of breast, colorectal carcinoma and lung carcinoma. In developing countries like India, cervical carcinoma accounts for the second most common cancer occurring in women and also the second leading cancer killer next only to carcinoma breast. Every year 528,000 new cases of carcinoma cervix are diagnosed. Of this, one-fifth of cases occur in India[1]. The most important etiological factors responsible for the development of cervical carcinoma and its precursor lesions are chronic infection with HPV (human papilloma virus)[2]. Invasive carcinomas of cervix usually progress from dysplasias over a period of several years. Hence screening programmes, if started early, can identify most lesions in their initial stages, even before the development of overt malignancy.

There are many well established prognostic factors for carcinoma of cervix including FIGO stage, HPV status, age of the patient, tumor size, depth of stromal invasion, nodal status,parametrial involvement,lymphovascular invasion etc. Recently, several biomarkers were studied which may have a role in predicting the prognosis and also in the selection of therapeutic strategies that can be helpful to further improve patient survival for carcinoma of cervix. Of these one important molecular marker is cycloxygenase 2 (COX2).

Cycloxygenase (COX) is a catalystic enzyme that aids in the formation of prostaglandin from arachidonic acid. Two distinct forms of COX exists – (1)COX-1, (2) COX-2. COX-1 is known as the housekeeping gene and have constant levels of expression in all cells. COX-2 is an immediate-early response gene that is activated by several factors liketumor promoter oncogenes, growth factors and carcinogens. It has been proven that COX-2 is overexpressed in malignant cells especially in colorectal neoplasms and carcinoma of cervix.

In our study, we evaluate thepattern of COX-2expression in cervical carcinomas and its association with various clinicopathological variables like age of the patient, stage, menstrual status, tumor size, parametrial involvement, vaginal involvement, nodal status and histological grade of tumor.

AIMS

AND

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- To assess the expression of Cycloxygenase 2 (COX-2) in invasive cervical carcinomas
- To compare the clinical behaviour of cervical carcinomas and cyclooxygenase 2 expression
- To correlate the expression of cyclooxygenase -2 (COX2) with various clinicopathological variables
- To assess the value of COX2 as a prognostic and predictive marker.

REVIEW

OF

LITERATURE

REVIEW OF LITERATURE

ANATOMY:[3,4]

The cervix (in Latin means neck) is the lowermost portion of uterus, that protrudes into the upper part of vagina. In an adult nulligravida, the cervix measures about 2.5 – 3 cm in length, and is positioned slightly downward and backward. Cervix is divided into two portions:

1. Portio vaginalis – the part that is protruding into the vagina
2. Supravaginal portion – the part that is lying above vaginal vault.

Ectocervix or exocervix is the outer part of portio vaginalis. The part related to endocervical canal is the endocervix. The opening of endocervical canal to the ectocervix is the external os& the upper limit of endocervical canal is the internal os. The portio vaginalis is divided into anterior and posterior lips.

The cervix is supplied by uterine arteries. Venous drainage parallels arterial system. Lymphatics of cervix collect into two lateral plexuses near the isthmus & gives rise to 4 efferent channels coursing towards external iliac nodes & obturator nodes, hypogasrtic & common iliac nodes, & nodes on posterior wall of urinary bladder. The cervix is innervated chiefly in the endocervix& peripheral deeper portions of ectocervix. This is the reason for the inner two third of portio vaginalis to be relatively insensitive to pain.

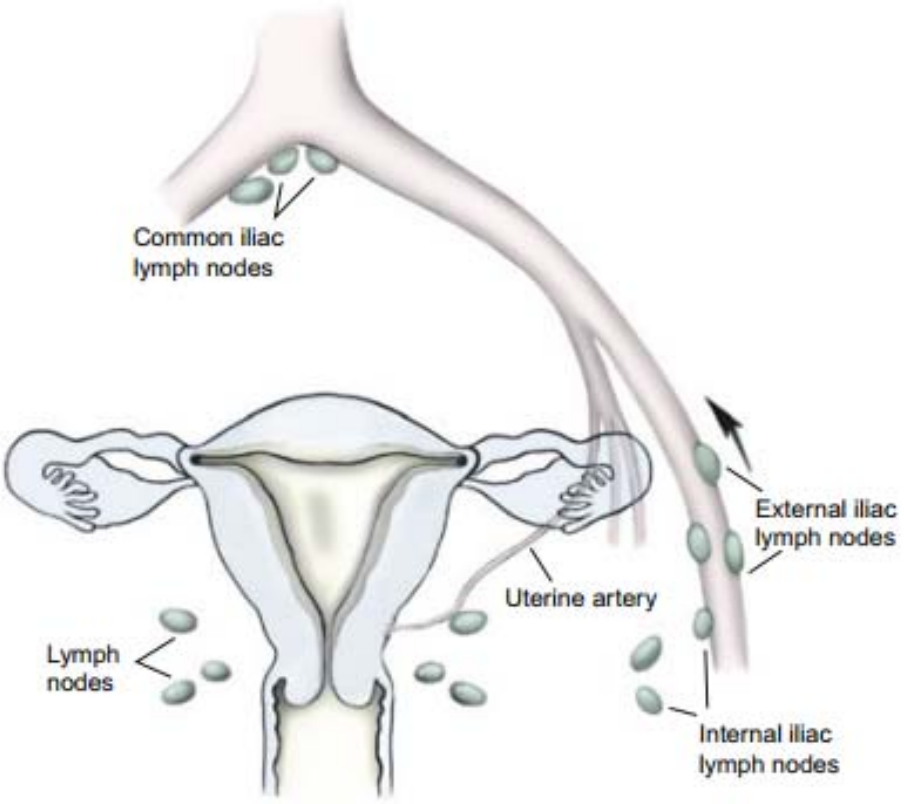


Figure showing thelymphatic drainage and blood supply of cervix

HISTOLOGY OF CERVIX:[3,4]

Cervix is made up of a mixture of fibrous, elastic and muscular tissue which is lined by columnar epithelium and squamous epithelium. Ectocervix is lined by mature non-keratinizing stratified squamous epithelium. It has 3 zones:

1. Germinal/ Basal/ Parabasal/ cell layer – helps in continuous epithelial renewal
2. Mid zone/ Stratum spinosum – the prominent part of cervical epithelium
3. Superficial zone – contains the most mature cells.

The lining of endocervix a single layer of columnar epithelial cells, which also line the underlying glands. These cells have finely granular cytoplasm which is filled with mucin and basally placed nuclei. Dendritic cells and lymphocytes are also seen in the epithelium and stroma of the ectocervix and endocervix.

Transformation zone is the region between original squamocolumnar junction & the functional/ physiologic/ new squamocolumnar junction. This zone is characterised histologically by the presence of metaplastic epithelium. Virtually all cervical squamous epithelial neoplasia arises at the transformation zone.

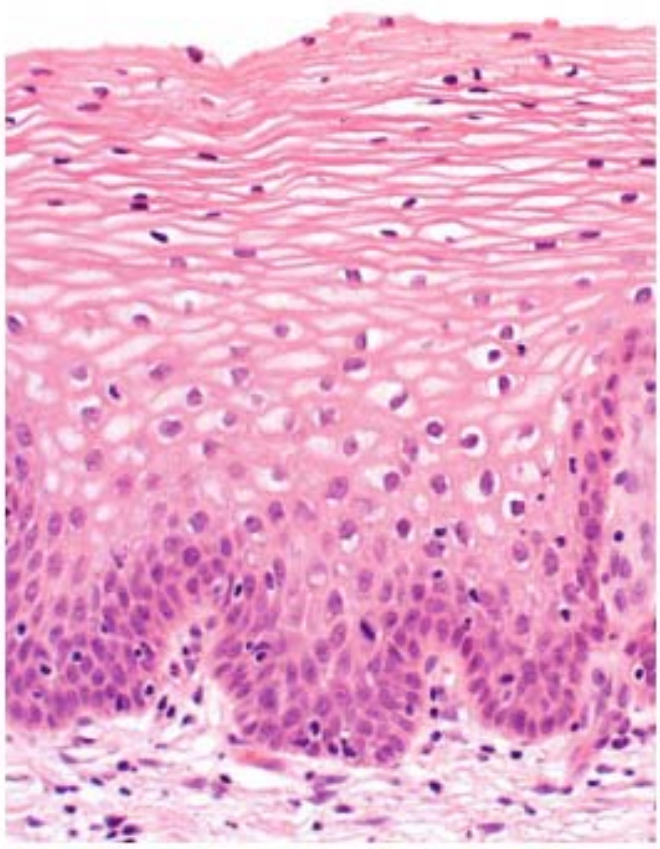


Figure showing normal ectocervical stratified squamous epithelium.

Cervical carcinoma is one of the leading causes for cancer related deaths in Indian women[5]. The most important etiological factors responsible for the development of carcinoma of cervix and its precursor lesions are chronic infection with human papilloma virus (HPV)[3]and also early age of first sexual intercourse[4].

It has been proven that carcinoma of cervix almost always is preceded by precursor lesions. In 1886, Sir John Williams recognised that areas adjacent to invasive squamous cell carcinoma showed non-invasive dysplastic changes[6]. Later, in 1990, Cullen found out that these non-invasive lesions resemble invasive carcinoma histologically[7]. These lesions were later studied in detail & were designated as cervical intraepithelial neoplasia (CIN) and are the precursor lesions of invasive cervical carcinoma.

This knowledge has led to the development of various screening procedures to detect the precancerous lesions at an early stage so that it can be treated effectively and thus preventing their progression to invasive cancer. With the effective use of these screening procedures carcinoma of cervix has now become one of the preventable causes of cancer morbidity and mortality. Today the incidence of carcinoma of cervix, predominantly the squamous cell type is showing a steady decline worldwide.

It is also anticipated that the widespread use of HPV vaccination will help in further reduction of the incidence of this cancer in near future.

SCREENING FOR CARCINOMA OF CERVIX

Screening for carcinoma of cervix includes:

- Cervical cytology – conventional (pap smear)
- Cervical cytology – liquid based preparation
- HPV testing
- Colposcopy – VIA, VILI
- Cervical biopsy
- Immunomarkers

Papaniculaou tests / Pap test was introduced as a screening method for carcinoma of cervixabout half a century ago. It was first done by George Papaniculaou[8]. Even today, it is considered as the most effective screening procedure for detection of a malignancy. Introduction of pap test has played a crucial role in decreasing the incidence of cervical carcinoma worldwide.

Pap test is a simple, less expensive, easy to perform, minimally invasive screening procedure that can be done as an out-patient procedure. Ayers spatula, which is a wooden spatula with bifid end, is used to take scrapings from the transformation zone of the cervix, which is then smeared on glass slides. These glass slides are immediately fixed in alcohol. The fixed slides are stained using Papanicolau stain and examined under the microscope.

Several advances have been introduced in cervical cytology techniques including the method of collection of sample, evaluation of cytological smears and classification system used for reporting these smears.

The conventional method of pap smear has been replaced recently by liquid based cytology. In this method, the sample is collected with the help of a cervical brush, which is transferred into a liquid fixation solution and sent to the laboratory. In the laboratory, the sample is centrifuged and the button is used to make an even smear which is stained and viewed under a microscope.

Previously, yearly screening was recommended for women of age 21 – 65 years. But according to latest guidelines by American Cancer Society[9]:

- Screening for carcinoma of cervix should be started at the age of 21.
- Women of age 21-29 years should undergo screening once in every 3 years
- Women in age group of 30 – 65 years should do both pap test and HPV test once in every 5 years, or a pap test alone in every 3 years.
- Women of age more than 65 years, who had regular screening with normal results need not be screened further.

The Bethesda system was introduced in 1988 to provide uniform guidelines for reporting cervical cytology. This was later revised in 1991 and 2001[10]. Terence J Colgan has discussed the various sections of the Bethesda system in 2001[11]. The Bethesda system 2001 is given in annexure No.1.

Testing for HPV infection is another important screening procedure for carcinoma of cervix. Most of the HPV infections are transient at 1-3 years interval and only persistent infection has the risk of progression to cervical

cancer. Risk of HSIL/cancer after 3 years is not significantly higher than the risk after 1 year. Hence 3 years interval can be given between screening visits.

HPV testing is not needed in the 21-29 year group because the prevalence of carcinogenic HPV in early 20's is 20%, most of which disappear without intervention. HPV testing in this age group creates unnecessary anxiety and intervention and hence avoided.

HPV co-testing is added in 30-65 year age group because of increased prevalence of CIN III in this age group. Addition of HPV co-testing also reduces the number of colposcopies. However this cannot be done in all areas because of financial restrictions and hence cytology alone every 3 years is also acceptable in this age group.

RISK FACTORS FOR CARCINOMA OF CERVIX[3,5]

The risk factors associated with the various precancerous lesions and carcinoma of cervix are:

- Infection with high risk subtypes of Human Papilloma Virus (HPV)[12]
- Multiple sexual partners
- Age at first sexual intercourse
- Lower age at first pregnancy
- Multi-parity
- Lower socioeconomic status

- Smoking[13]
- Immunosuppression
- Human immunodeficiency virus
- Sexually transmitted disease
- Nutritional deficiency
- Use of oral contraceptive pills
- Inadequate screening

Of all the above, the most important ones are infection with high risk subtypes of HPV and inadequate cervical cancer screening.

The presence of other risk factors like immunosuppression, smoking, long term OCP use in women with high risk types HPV infection doubles or triples the risk for development of high grade squamous intraepithelial lesion and invasive malignancy[13].

The positive association between cigarette smoking and development of cervical carcinoma has been reported in several studies[14].

A meta-analysis of 28 studies on the association of oral contraceptive pill use and cervical cancer development demonstrated that relative risk for invasive cervical carcinoma increased with increasing duration of OCP use[15].

Studies have proven the role of nutritional deficiency, especially of micronutrients like retinol, folate, vitamin E in cancer development[16].

Immunosuppression, which may be due to HIV infection, or post organ transplant or due to acquired or congenital immunodeficiency syndromes is a

well-established risk factor for development of cervical intraepithelial neoplasia as well as invasive cervical carcinoma.

Low socio economic status is associated with poor hygiene, early sexual intercourse, micronutrient insufficiencies, increased comorbid conditions, which all are proven risk factors for the development of cancer cervix.

ASSOCIATION OF HPV WITH CARCINOMA OF CERVIX

Carcinoma of cervix is unique among human cancers because it is the first carcinoma found to be directly attributable to the effects of an infectious agent. This was first discovered by Dr. Herald zurHausen, for which he was awarded the Nobel Prize in medicine in 2008[17].

Human papilloma virus belong to the family Papillomaviridae[18]. Papillomaviruses are epitheliotropic viruses, which means they infect predominantly the skin and mucous membranes producing epithelial proliferations at the site of infection. They are double stranded DNA viruses. There are 118 distinct types of papilloma viruses[17]. HPV, in addition to cervix cancer, is also known to be associated with vulval, anal, penile and some of the head and neck squamous cell carcinomas[19].

A study done to detect the incidence of HPV in cervical carcinomas by multiple molecular techniques detected that the prevalence of HPV in cancer cervix across the world is 99.7%[20]

The HPV types that are associated with invasive carcinoma of cervix include HPV 16, 18, 31, 35, 33, 39, 45, 51, 56, 58, 59, 66 and 52. Among these, the most commonly associated HPV type is HPV 16, followed by HPV 18[21].

The initial site of HPV infection is the basal cell of the ectocervical epithelium. HPV can reside in the epithelial cells in two different biological forms. One is latent infection, in which no infectious virions are produced and the other is productive infection, in which numerous infective virions are produced.

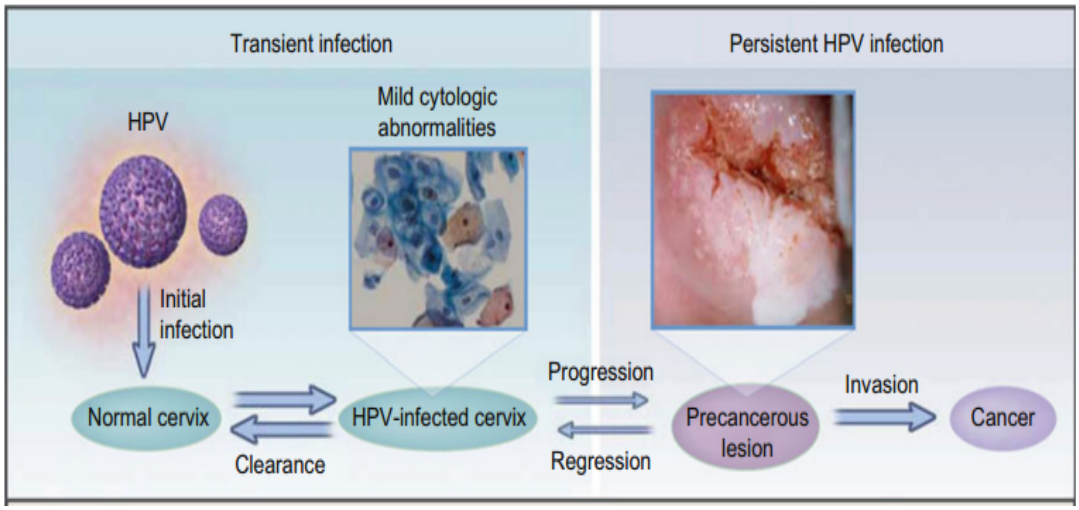


Figure showing the effect of HPV infection in cervical epithelium

The cytological features of virus infected cells include:

- Koilocytosis
- Acanthosis
- Anisocytosis
- Perinuclear cytoplasmic vacuolization
- Multinucleation
- Nuclear atypia.

PRECURSOR LESIONS OF SQUAMOUS CELL CARCINOMA OF CERVIX

The 3 different systems of classification of precursor lesions are compared in the following table.

Older classification	**WHO classification**	**Bethesda system**
Mild dysplasia	CIN 1	Low grade squamous intraepithelial lesion (LSIL)
Moderate dysplasia	CIN 2	High grade squamous intraepithelial lesion (HSIL)
Severe dysplasia/ Carcinoma in situ	CIN 3	HSIL

The term cervical intraepithelial neoplasia (CIN) was introduced by Richart who proposed that all the precursor lesions of cervical carcinoma are monoclonal proliferations and represent a single disease process[22]. According to him, CIN 1 is synonymous to mild dysplasia, CIN 2 to moderate dysplasia and CIN 3 to severe dysplasia/ carcinoma in situ.

Later it was proved that the invasive carcinoma of cervix (squamous and adenocarcinoma) are caused by infection of anogenital tract by high risk types of HPV[23,24]. Hence a new model of cervical carcinogenesis was developed[25,26,27], which involves:

1. Infection with high risk types of HPV
2. Progression to precursor lesion
3. Invasion

Subsequently, the new Bethesda system was introduced which categorised the lesions into two (Two tier system)[28,29]:

1. LSIL (Low grade squamous intra epithelial lesion) – for those lesions initially called CIN 1.
2. HSIL (High grade squamous intra epithelial lesion) – for those lesions initially called CIN 2 & CIN 3

CLINICAL FEATURES

- Early invasive cancers are usually asymptomatic and are detected during a routine screening by pap smear.
- As the tumor increases in size, patient may complain of irregular vaginal bleeding, metrorrhagia, or discharge per vaginum.
- Another common presenting symptom is dyspareunia (pain during sexual intercourse)
- When the tumor extends to the parametrium, it can cause obstruction of the ureters causing hydronephrosis, anuria, or uremia.
- Pelvic side wall extension of the tumor causes sciatic pain or less frequently lymphedema of lower extremities.
- Bladder involvement of the tumor can cause increased frequency of micturition, hematuria, bladder pain, urinary retention or vesicovaginal fistula.
- Rectal infiltration by tumor can cause pain, bleeding per rectum, rectovaginal fistula.
- Patient may sometimes present with symptoms of distant metastasis like cervical node enlargement, hemoptysis etc.
- Non-specific symptoms like weakness, pallor, weight loss may also be the presenting symptoms in some patients.

DIAGNOSIS

Diagnosis of cervical carcinoma is made combining the findings of history, physical examination, imaging and biopsy.

- History – history of irregular vaginal bleeding, post coital bleeding or post-menopausal bleeding can suggest carcinoma of cervix in a multiparous women from a lower socioeconomic status.
- General examination to look for pallor, lymph node enlargement or any signs of metastatic disease.
- Speculum examination to look for any growth in the cervix, type of growth (ulcerative / nodular / papillary), bleeding on touch.
- Per vaginal examination to look for the extend of growth, parametrial involvement, vaginal involvement, seedling in pouch of Douglas, involvement of ovary
- Pap smear – to look for malignant cells in cervical cytology
- Per rectal examination – to look for rectal involvement in advanced diseases.
- Cystoscopy – to look for bladder mucosal involvement by the tumor.
- Human Papilloma Virus (HPV) testing – to detect the specific viral DNA or RNA.
- Endocervical curettage – to detect the presence of malignant cells.

- Ultrasound, CT, MRI – to look for extend of parametrial invasion, ureteric involvement, hydrouretronephrosis, lymph node involvement, bowel and bladder extension of tumor.
- Chest X-ray, CT chest – to look for evidence of lung metastasis
- Biopsy from cervix – this is the confirmatory test for the diagnosis of carcinoma of cervix. Biopsy can also detect the histological type of carcinoma.

CLINICAL STAGING OF CARCINOMA OF CERVIX

Carcinoma of cervix is unique in that the staging is done clinically. The various parameters for staging include:

- Size of the tumor
- Parametrial involvement
- Involvement of vagina
- Presence/absence of hydronephrosis or non-functional kidney.
- Bladder or rectal involvement
- Presence/absence of distant metastasis

Clinical staging is done by:

- Physical examination
- Ultrasound
- Intravenous pyelogram
- Cystoscopy

- Proctoscopy
- Chest X-ray

The presence of lymph node metastasis or margin involvement detected after surgery by histopathological examination will not alter the stage.

GROSS:

- Early lesions – focal induration, ulceration or a nodular lesion, mostly localized within the transformation zone.
- Advanced lesions may be endophytic or exophytic.
- Endophytic lesions may be ulcerated or nodular, and usually invade deep into the cervical stroma producing a barrel shaped cervix.
- Exophytic lesions have a polypoidal, fungating or papillary appearance.

WHO classifies invasive cervical carcinoma broadly into:

- Squamous cell carcinoma
- Adenocarcinoma
- Other epithelial tumors
    - Adenosquamous carcinoma
    - Neuroendocrine tumors
    - Un-differentiated carcinoma

INVASIVE SQUAMOUS CELL CARCINOMA OF CERVIX

The commonest histologic subtype of carcinoma of cervix is squamous cell carcinoma and accounts for 70-80% of all cervical carcinomas[30]. The most important etiological factor responsible for development of carcinoma of cervix is infection with high risk subtypes of HPV, especially HPV 16 and HPV 18[31]. The other types of HPV implicated in the cervical carcinogenesis include HPV 31, 33, 35, 45, 52 & 58[32].

The most commonsymptoms of patients having an invasive cervical carcinoma are abnormal vaginal bleeding and post coital bleeding. Other symptoms include intermittent spotting, serosanguinous discharge, pain, weakness, weight loss, rectal pain or hematuria.

Grossly, early carcinomas present as localized growth within the transformation zone, whereas advanced cases present as exophytic or endophytic tumors that invade into cervical stroma and into the adjacent structures.

Microscopically SCC is characterized by the anastomosing cords and tongues of neoplastic squamous epithelial cells infiltrating the stroma. Current WHO classification divides invasive SCC into 2 groups:

1. Keratinizing SCC – keratin pearls are present within the epithelium
2. Non- keratinizing SCC – neoplastic cells with individual cell keratinization &no keratin pearl formation.

The grading system for SCC was proposed by Broders in 1920[33]. The currently used grading system is a modification of the original form and consists of 3 groups:

1. Well differentiated SCC (Grade 1) –Characterised by abundant keratin, deposited in the form of keratin pearls in between neoplastic epithelial cell nests. The cells have abundant eosinophilic cytoplasm, large, irregular and hyperchromatic nuclei and well developed intercellular bridges. Mitotic figures are seen predominantly near the periphery of advancing epithelial cell nests.
2. Moderately differentiated SCC (Grade 2) - Neoplastic cells show more cellular pleomorphism than grade 1 tumors with less abundant cytoplasm and large irregular nuclei. Intercellular bridges and cellular borders appear indistinct. Here keratin pearl formation is seen only rarely, but there will be individual cell keratinization present towards the centre of tumor cell nests. More numerous mitotic figures are noted than in grade 1 SCC.
3. Poorly differentiated SCC (Grade 3) - composed of cells with scant indistinct cytoplasm and hyperchromatic oval nuclei. Definite evidence of squamous differentiation manifested by intercellular bridges or keratinization are difficult to find. Abundant areas of necrosis and numerous mitotic figures are seen.

The treatment options for SCC of cervix include surgery, radiation, chemotherapy or combinations of these 3.

ADENOCARCINOMA OF UTERINE CERVIX

Adenocarcinoma includingadenosquamous carcinoma comprises 10-15% of all cervical malignancies[34]. Though the incidence of cervical carcinoma is decreasing worldwide, many studies reveal the increasing rate of adenocarcinoma in young women[35,36,37].

Most of the risk factors for cervical adenocarcinoma are similar to that ofSCC[38,39]. HPV 18 is more commonly associated with cervical adenocarcinoma than with squamous cell carcinoma[40,41].

Majority of cervical adenocarcinomas arise in the transformation zone. Patients usually present with vaginal discharge, abnormal vaginal bleeding or pelvic pain.

Grossly the lesion may be a fungating, polypoidalor papillary mass or a diffusely enlarged or nodular cervix.

Microscopically, the most common histologic type is the usual type of cervical adenocarcinoma, which comprises more than 75% of cervical adenocarcinomas. The cells are columnar in shape having amphophilic or eosinophilic apical cytoplasm and elongated, hyperchromatic, pleomorphic nucleuswith coarse chromatin. Mitotic figures and apoptotic bodies are frequent.

Histological grading of adenocarcinoma is:

- Well differentiated ADC – less than 10% of tumor is formed by solid sheets of cells.
- Moderately differentiated ADC– 11 – 50% of tumor is formed by solid sheets of cells.
- Poorly differentiatedADC – more than 50% of tumor is made up of solid sheets of cells.

True endometrioid-type endocervical adenocarcinoma accounts for about 10% of all cervical adenocarcinomas.

Mucinous Adenocarcinoma can be of 3 types:

1. Endocervical-Type Mucinous ADC
2. Intestinal-Type Mucinous ADC
3. Signet-Ring Cell-Type Mucinous ADC

The term 'minimal deviation adenocarcinoma' was introduced in 1975 by Silverberg and Hurt [42].It is an extremely well - differentiated variant of cervical adenocarcinoma.Here most of the tumor cells lack cytologic features of malignancy. These account for 1–3% of all cervical adenocarcinomas.

Villoglandular adenocarcinoma is another well-differentiated type of cervical adenocarcinoma occurring predominantly in young women and is associated with an excellent prognosis[43,44].

Endometrioid adenocarcinomas of the cervix resemble primary adenocarcinomas of uterine corpus. The cells of endometrioid adenocarcinomas are stratified and have oval nuclei with lesser amount of cytoplasm than the cells of usual type of endocervical adenocarcinomas.

Clear cell carcinomas comprise around 4% of cervicaladenocarcinomas .Postmenopausal women are more commonly diagnosed with this variant. Most of the clear cell carcinomas of cervix diagnosed during the period from 1970 to late 1990s were related to exposure to diethylstilbestrol (DES) prenatally and were more common in younger women[45,46]. Three basic microscopic patterns are seen in clear cell carcinoma namely solid, tubulocystic and papillary patterns.Tumor cells have well defined cell margins, moderate to abundant clear cytoplasm (due to accumulation of glycogen) and high grade nuclei. The nuclei may be seen projecting into the lumen of cysts and tubules forming 'hobnail cells'.

ADENOSQUAMOUS CARCINOMA

These tumors are composed of an admixture of malignant glandular and squamous cells. Adenosquamous carcinomas comprises 3.6% to 25% of all cervical cancers[47,48,49,50]. The squamous component are usually well differentiated with keratin pearl formation or cells with individual cell keratinization.Adenocarcinomatous component may or may not be well differentiated, and if present in relatively small amounts, can easily be overlooked. A recent study have shown that the squamous and glandular components are monoclonal in origin and they originate from a common

precursor cell[51]. Metastasis to pelvic lymph nodes is twice more common in adenosquamous carcinoma than in pure adenocarcinomas or squamous cell carcinomas. Despite this, the prognosis is not significantly worse than those with squamous cell carcinomas.

Glassy cell carcinoma is poorly differentiated variant of adenosquamous carcinoma and accounts for less than 1% of all cervical cancers. Grossly they produce a barrel-shaped cervix. Microscopically they are composed of large uniform polygonal cells with distinct cell membranes, ground glass-type cytoplasm, and prominent nucleoli. The stroma is characteristically heavily infiltrated by lymphocytes, plasma cells and eosinophils. These are extremely aggressive tumors and response to radiation & surgery are very poor.[52,53].

PROGNOSTIC FACTORS

- Clinical criteria:[54,55,56]
    - Age
    - Stage of the disease
    - Lymphatic spread
    - Vascular invasion
- Histopathological criteria:[57,58,59]
    - Tumor size
    - Depth of invasion
    - Parametrial invasion
    - Nodal status
    - Lymphovascular invasion

- Human papilloma virus DNA status
- Genetic predictive factors
    - c-erb B
    - c-MYC

Younger women are found to have a better prognosis when compared to older women[60].

Stage of the disease is the most important prognostic factor for carcinoma of cervix. The stage, in turn, is depended on size of tumor, depth of invasion, parametrial infiltration and nodal status.

Several studies have proved that the 5 year survival rate of patients with smaller tumors and less of invasion into the cervical stroma is significantly increased than those with larger tumors& more invasion[61].

In patients with lymphovascular invasion, the 5 year survival rate was found to be decreased when compared to those with no lymphovascularinvasion[59]. Also, the presence of lymphovascular invasion was found to be associated with 4 -5 times more risk of lymph node metastases[57], and hence a decreased 5 year survival rate[62].

The parametrial involvement by tumor is associated with lymph node metastasis, local recurrence & poor survival rate[61,63].

Studies assessing the effect of HPV types on prognosis have shown that detection of HPV 18 DNA was associated with a poor prognosis and increased risk of death irrespective of the presence or absence of other prognostic

factors[64,65]. Studies have also shown that those patients who are infected with multiple HPV types are more resistant to radiation treatment when compared to those infected with a single HPV type[66,67].

Amplifications of c-erb B-2 and c-myc are found to be associated with poor prognosis[68,69].

PROGNOSTIC BIOMARKERS

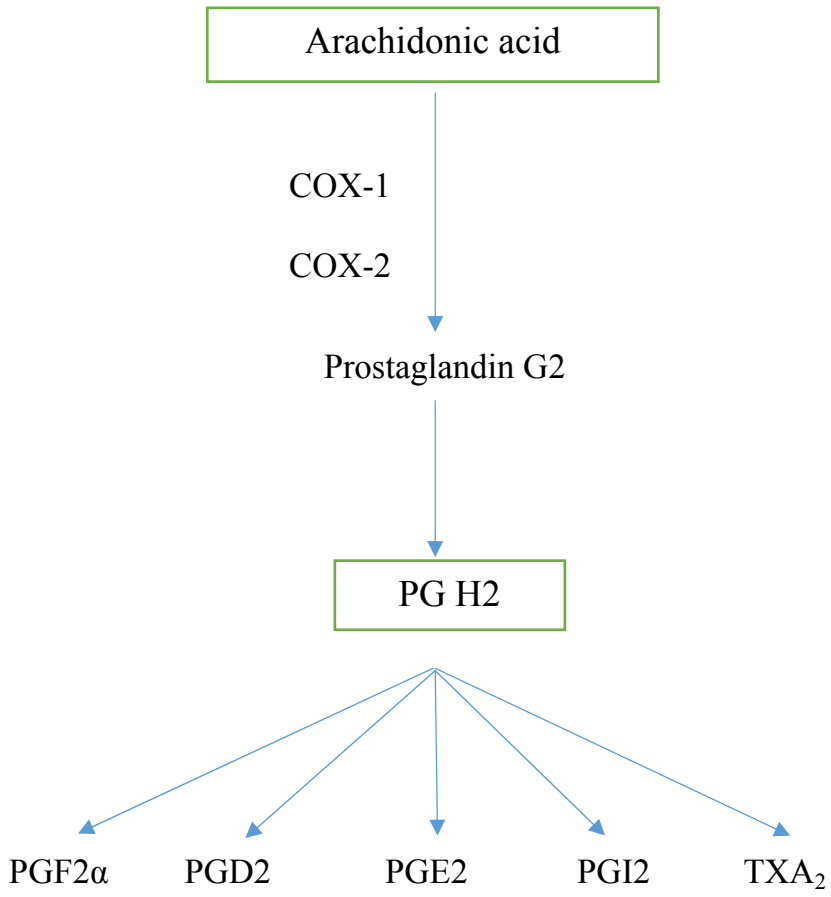
The role of immunohistochemistry in detection of various prognostic biomarkers in cervical carcinoma has been studied extensively. The various biomarkers that have been studied so far include

- Ki – 67
- LRIG1
- COX – 2
- EGFR
- HER 2
- VEGF
- D2-40
- Hyaluronan receptor – 1

ROLE OF COX-2 IN CERVICAL CARCINOMA

Cyclooxygenase (COX), is an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins, thromboxane &levuloglandins[70]. It is also designated as prostaglandin endoperoxide synthase (PES).COX was first identified about 30 years ago. Two isoforms of COX has been identified.

1. COX-1 – produced constitutively in many tissues and is referred to as the house keeping gene.
2. COX-2 – inducible enzyme synthesised in sites of inflammation[71].



COX-2 is an inducible gene or an early response gene. The various factors that induce COX2 expression include various growth factors, oncogenes, prostaglandins, tumor promoters and carcinogens[72]. COX-2 overexpression has been well documented in colorectal carcinomas[73]. High COX-2 expression has also been demonstrated in various other lesions like high grade squamous cell carcinoma of oesophagus[74], pre-neoplastic lesion of lung and pre-invasive lesions of breast[75], adenomatous and metaplastic lesions of stomach[76], pre-invasive neoplasms of bladder[77] and pancreas[78].

COX 2 increases local prostaglandin production in tumor cells and induces tumorigenesis by affecting cellular adhesion, mitogenesis, apoptosis, angiogenesis and immune surveillance. Studies have proven the fact that COX2 inhibits apoptosis.

Studies on the expression of COX-2 in carcinoma of cervix are relatively few. Sukumvanich et al[79] and Kulkarni et al[72] have shown that the expression of COX-2 increases with increasing severity of dysplasia to invasive carcinoma. Also high COX-2 expression was found to be associated with parametrial invasion &lymph node metastasis in stage IBtumors[80]. High COX-2 expression also correlated with worse prognosis in terms of overall survival & disease free survival in patients treated with radiotherapy[81]. A positive association of presence of lymphovascular emboli & COX-2 expression have also been demonstrated[82]. These studies signify the role of COX-2 as a prognostic marker.

However, some studies have failed to demonstrate a significant correlation between COX-2 expression and tumor response in post radiotherapy patients[83].

Kulkarni et al demonstrated that EGFR and its ligands EGF & TGF-α markedly induce COX-2 in cervical cancer, which suggest that deregulated signalling through EGFR may account for the increased COX-2 expression. EGF exerts its action by stimulating RAS & P13 signalling, which in turn leads to activation of MAPKs and an increased AP-1 activity that are responsible for induction of COX2[84].

In a meta-analysis of 23 studies with 1477 cervical carcinoma patients, it was found that COX-2 over expression was associated with an unfavourable prognosis and correlated with chemoradiation resistance in cervical carcinoma, which could potentially help to stratify patients for further treatment options[85].

Studies using selective COX2 inhibitors (celecoxib) in patients with carcinoma of cervix have shown a reduced expression of COX-2, CD31 & Ki-67 and a decrease in microvesseldensity[86]. But clinical trials using celecoxib in patients with advanced cervical carcinoma have failed to demonstrate any therapeutic effect[87,88].

OTHER BIOMARKERS IN CERVIX CARCINOMA

1. **Ki-67** – studies have shown that high Ki-67 index intumor cells was associated with poor prognosis inlate stage cervical carcinoma[89].
2. **LRIG1**(Leucine rich repeats & immunoglobulin like domains-1) – expression of this transmembrane protein was associated with favourable prognosis in patients with early stage cervical carcinoma[89]. LRIG1 was shown to exert its effects by restricting growth factor signalling and degrading the EGFR and thereby inhibiting the cellular oncogenes MET & RET.
3. **EGFR** – the expression of EGFR in cervical carcinoma is found to be associated with a poor prognosis[90]. EGFR acts y promoting RAS & P13K signalling, leading to activation of MAPKs, which in turn induce the expression of COX-2.
4. **HER-2** (EGFR-2) – HER-2 expression is also found to be associated with a poor prognosis.
5. **D2-40**(marker for lymphatic vessel endothelium) – Expression of D2-40 which correlates with peritumoral lymphatic vessel density has shown a significant correlation with lymph node metastasis, high tumor stage & poor survival[91].
6. **Hyaluronan receptor-1** (which is also an antibody against lymphatic vessel endothelium) – increased expression of this antibody was associated with lymphatic vessel invasion & lymph node metastasis in early stage cervical cancer[92].

7. **VEGF** (Vascular Endothelial Growth Factor) – overexpression of VEGF is associated with poor prognosis in many cancers, including cervical cancer. High VEGF expression correlates with increased risk for lymph node metastasis and high stage of disease[93]. High VEGF expression & increased tumor vascularization are demonstrated to be independent predictors of shorter disease-free survival & overall poor survival[94].

TREATMENT

The treatment of cervical carcinoma depends on the stage of disease. The various treatment options include the following:

- Carcinoma in situ / CIN III – local ablation or excisional procedures like cryosurgery, laser excision, loop electrosurgical excision procedure (LEEP). If family is completed, then simple hysterectomy is usually recommended.
- Stage IA1 – simple / radical hysterectomy without radical lymph node dissection. For young patients who would like to retain fertility can be treated with conisation procedures with close follow up using pap smears, colposcopy and endocervical curettage.
- Stages IA2, IB, IIA – treatment options include radical hysterectomy with bilateral pelvic lymph node excision, or combination of external beam radiation with brachytherapy. For stage IA2 patients who wish to retain fertility, radical vaginal trachelectomy can be done along with pelvic

lymph node dissection. For patients with positive nodes or positive surgical margins, post-operative radiation can be given.

- Stages IIB, III, IVA – for these locally advanced diseases, treatment options include radiotherapy or combined radiation and chemotherapy (cisplatin based)
- Stage IVB and recurrent carcinomas – palliative therapy. For pain and vaginal bleeding, radiotherapy can be given. For disseminated diseases, chemotherapy is given. For local recurrences, chemoradiation is the usual treatment option, whereas for recurrence outside pelvis, combination chemotherapy is usually recommended.
- Cervical carcinoma in pregnancy – carcinoma of cervix is the most common gynaecological carcinoma in pregnancy. For very early stage carcinomas, pregnancy can be continued, and after delivery specific treatment for carcinoma can be given. For advanced cases, it is better to terminate the pregnancy and treat the malignancy. If the patient wishes to continue with her pregnancy, then pregnancy should be terminated by caesarean section as soon as the baby becomes viable.

IMMUNOHISTOCHEMISTRY (IHC)

IHC is a molecular technique which was first described by Dr.Albert Coons in 1941. The original method consisted of an antibody developed in rabbits and then tagged with a fluorescent probe. It was mixed with tissue

sections and examined using a fluorescent microscope after a period of incubation. Since then, numerous advancements have been made[95].

The most commonly used techniques are the peroxidase -antiperoxidase immune complex method developed by Sternberger in 1970 and the biotin avidinimmunoenzymatic technique developed byHeitzman and Richards in 1974[96,97].

ANTIGEN RETRIEVAL

Shi et al in 1991developed the antigen retrieval technique, in which he used a heating method at high temperatures to bring out the antigenicity of the tissue which had been masked by formalin fixation.

Antigen retrieval can be done by proteolytic induced epitope retrieval or heat induced epitope retrieval

HEAT INDUCED EPITOPE RETRIEVAL

In this technique, tissue sections are placed in the retrieval solution which is heated for varying period of time that leads to the breakdown of protein cross-links which are formed during fixation with formalin and recovers the tissue antigenicity[98].

Commonly used heating devices are the pressure cooker, microwave oven, autoclave, steamer and water bath. Heating is usually done for about

20 minutes followed by 20 minutes of cooling. The retrieval solution commonly used is the Citrate buffer with pH 6.0.

PROTEOLYTIC INDUCED EPITOPE RETRIEVAL[99]

Proteases liketrypsin, proteinase K, chymotrypsin and pepsin are usedfor restoring the tissue antigenicity. However, the limitation of this technique is thatsome epitopes are destroyed during this process and therefore alter the tissue morphology.

TARGET ANTIGEN DETECTION METHODS

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are the direct and the indirect methods. The direct method is a one -step staining procedure in which a labelled antibody directly reacts with the antigen in the tissue sections. Most commonly used labels are fluorochrome, horse radish peroxidase and alkaline phosphatase. Although this method is simple, rapid, and uses only one antibody, the sensitivity is lower. This is because signal amplification is less, and therefore it is not as commonly used when compared to the indirect methods.

In the indirect method, first layer is formed by an unlabelled primary antibody which binds to the target antigen. Then, a second layer is formed by using a labelled secondary antibody that reacts with the primary

antibody. This technique is more sensitive than the direct method because of better signal amplification. This is due to the binding of several secondary antibodies with conjugated fluorochrome to each primary antibody. Another advantage with this method is that it uses only a small number of secondary antibodies[100].

MATERIALS AND METHODS

MATERIALS AND METHODS

This study is a retrospective descriptive study of invasive cervical cancer conducted in the Department of Pathology, Institute of Obstetrics and Gynaecology, Egmore, Chennai during the period from January 2014 to December 2014.

A total of 3427 specimens were sent to the Department of Pathology, Institute of Obstetrics and Gynaecology, Egmore, Chennai during the period of Jan 2014 to Dec 2014 for histopathological examination.

Out of that 511 cases were cervix cancer, which includes 12 Werthims hysterectomies and 499 cervix biopsies. Among those, 50 cases were selected for our study. Among those 50 cases, 9 cases were hysterectomies and 41 were cervix biopsies.

SOURCE OF DATA

The cervix carcinoma cases diagnosed at the Department of Pathology, Institute of Obstetrics and Gynaecology, Egmore, Chennai during the period of Jan 2014 to Dec 2014, which have been sent by the Department of Gynaecology.

INCLUSION CRITERIA:

1. All Werthims hysterectomy specimens of cervical carcinomas.
2. All invasive cervical carcinomas, including squamous cell carcinomas, adeno carcinomas, adenosquamous carcinoma, clear cell carcinoma and undifferentiated carcinoma.
3. All small biopsy specimens of cervical lesions, proved histopathologically as malignant.

EXCLUSION CRITERIA:

1. All small biopsy specimens of cervical lesions, proved histopathologically as benign or non-neoplastic lesions.
2. All Werthims hysterectomy specimens with no evidence of residual tumor.
3. Post radiotherapy or chemotherapy specimens.
4. Recurrent tumors.

METHOD OF DATA COLLECTION

For the 50 patients included in the study, detailed history regarding age, presenting symptoms, parity and menstrual status were obtained. Patients were examined clinically for pallor, lymphadenopathy and mass per abdomen. Details of per speculum, per vaginal and per rectal examinations were noted.

Reports of cystoscopy, ultrasound abdomen & pelvis, computerised tomography of abdomen & pelvis were also noted.

Tissues were fixed in 10% neutral buffered formalin, processed and embedded in paraffin. 4 μm thick sections of the paraffin tissue blocks were cut and stained with eosin and hematoxylin. Slides were collected from slide filing and were reviewed. The original diagnosis of Invasive malignancy with Histological Grading according to WHO criteria for classification was made by the Senior Pathologist in almost all cases.

Tumours were sub typed as adenocarcinoma if it has features of gland formation, squamous cell carcinoma if it has features of keratinazation or intercellular bridges, adenosquamous carcinoma if it had features of both adeno and squamous cell carcinomas, clear cell carcinoma if they had cells with abundant clear cytoplasm, hyperchromatic and pleomorphic nuclei that project into the lumen of cysts and tubules to form 'hobnail cells', and undifferentiated carcinoma if no specific differentiating features are present.

50 cases of carcinoma of cervix were selected randomly and included for further evaluation and comparing the clinical and morphological parameters and to study the efficiency of marker (COX2).

IMMUNOHISTOCHEMICAL EVALUATION:

Immunohistochemical analysis of marker COX2 was done in paraffin tissue blocks using super sensitive HRP polymer system based on non-biotin polymeric technology. Sections of 4µ thickness were cut from the paraffin tissue blocks. They were transferred to gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with mouse monoclonal antibody (PathnSitu) against COX2 protein and then detected by the addition of secondary antibody conjugated with horse radish peroxidase – polymer and Diaminobenzidine substrate. The step bystep procedure of Immunohistochemistry is given in Annexure

Antigen	Vendor	Species	Dilution	Positive control
COX2	PathnSitu	Rabbit	Ready to use	Carcinoma colon

CONTROL:

Carcinoma of colon was taken as internal control for assessing COX2 reactivity & to avoid false negative results.

INTERPRETATION AND SCORING SYSTEM

The immunohistochemically stained slides were analysed for the presence of reaction, cellular localization (cytoplasm and cytoplasmic membrane) and percentage of cells stained.

The immunohistochemical expression of COX-2 was graded as:

Negative – no staining

1+ - less than 10% of tumor cells staining positive for COX-2

2+ - 10 -50% of tumor cells staining positive for COX-2

3+ - more than 50% of tumor cells staining positive for COX-2

In this study, for evaluation of COX-2 greater than 10% expression of the tumour marker in cytoplasm and cytoplasmic membrane of tumour cells (2+ and 3+) is considered as positive. Cases with no areas of positive staining (Negative) and with less than 10% staining (1+) are considered negative.

STATISTICAL ANALYSIS

Immunohistochemical analysis was done in paraffin embedded tissue samples using the statistical package for social science software version IBM SPSS version 20 which consisted computing the frequency counts and percentages for qualitative variables and mean for quantitative variables. The correlation between COX2 and various clinicopathological parameters was made and strength of association calculated using Pearson Chi square test. P values less than 0.05 are considered statistically significant.

OBSERVATION

AND

RESULTS

OBSERVATION & RESULTS

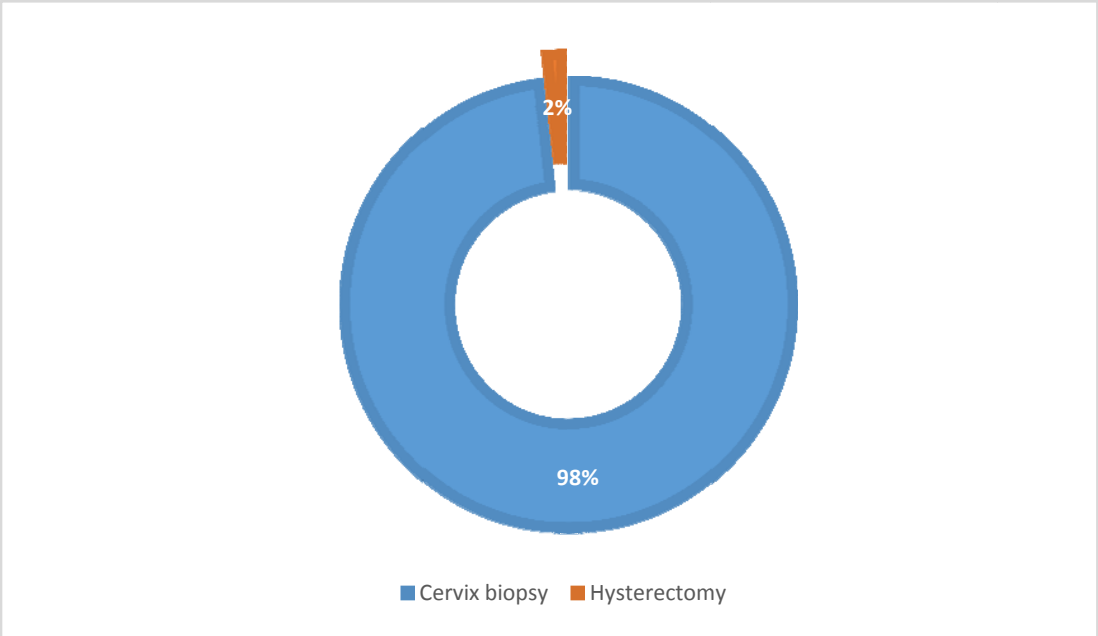
In the study period of 12 months from January 2014 to December 2014 a total of 3427 specimens were received in the Department of Pathology, Institute of Obstetrics and Gynaecology, Egmore, Chennai.

Total number of cervix carcinoma cases were 511 which accounts for about 14.91% of all cases. Of these, hysterectomy cases were 9 and small biopsies were 502.

Table 1 : Type of cervix specimens

Cervix biopsy	502	98.24%
Hysterectomy	9	1.76%
Total	511	100%

Chart 1 : Type of cervix specimens



Among the 502 small biopsies, 466were squamous cell carcinoma, 27 were adenocarcinoma, 5 were undifferentiated carcinoma, and one each of clear cell carcinoma of cervix, malignant mixed mulleriantumor of cervix, poorly differentiated carcinoma and adenosquamous carcinoma. The squamous cell carcinoma and adenocarcinoma were further subcategorised into well, moderate and poorly differentiated grades.

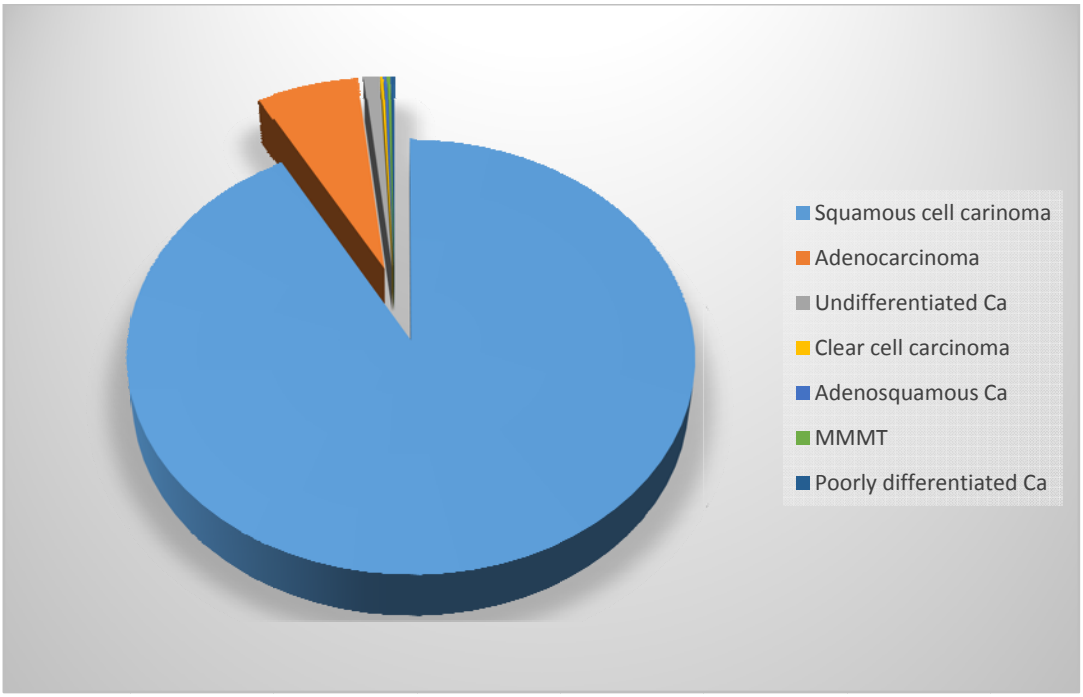
Among the 9 hysterectomies, 3 were moderately differentiated squamous cell carcinoma, 3 were poorly differentiated squamous cell carcinoma and 3 were well differentiated adenocarcinoma.

The most common type of carcinoma of cervix was squamous cell carcinoma (92.36%), followed by adenocarcinoma (5.87%)

Table 2: Histological subtypes of cervix carcinoma

Histological subtypes	**No. of cases**	**Percentage**
Squamous cell carcinoma	472	92.36%
Adenocarcinoma	30	5.87%
Undifferentiated carcinoma	5	0.97%
Clear cell carcinoma	1	0.19%
Adenosquamous carcinoma	1	0.19%
Malignant mixed mulleriantumor	1	0.19%
Poorly differentiated carcinoma	1	0.19%

Chart 2: Histological subtypes of cervix carcinoma

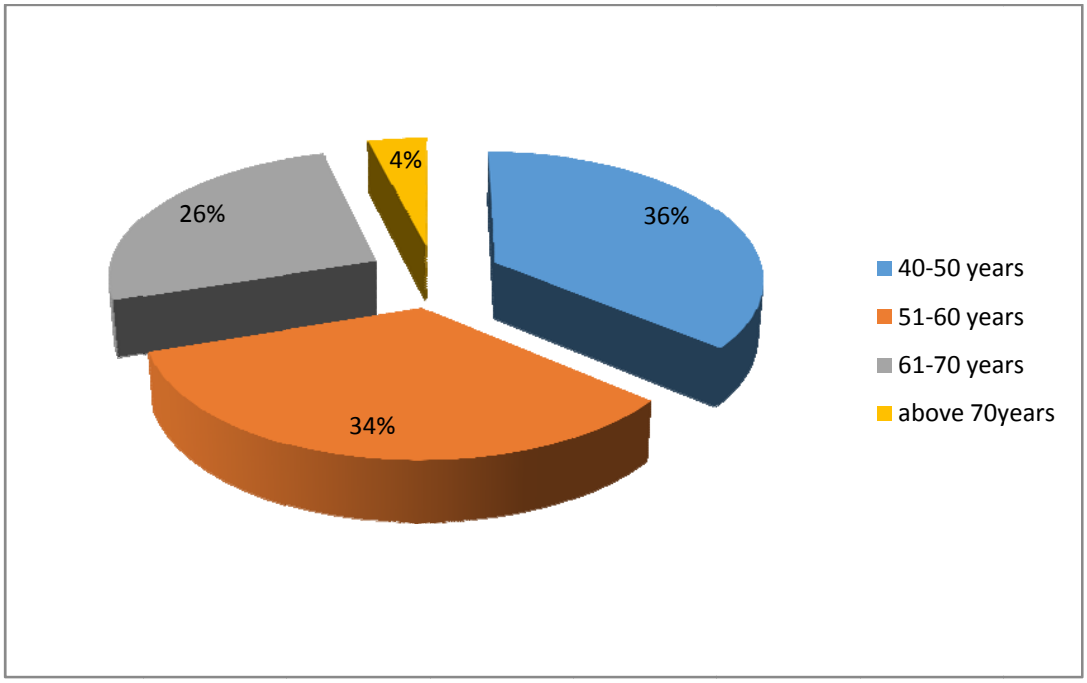


In the present study, the age of patients with carcinoma of cervix range from 40 – 80 years with mean age of 55.62 years. Maximum number of patients (36%) were of the age group 40-50years, closely followed by 51- 60 years age group (34%).

Table 3: Age wise distribution of carcinoma of cervix

Age group	No. of cases	Percent
40 - 50	18	36
51 - 60	17	34
61 – 70	13	26
Above 70	2	4
Total	50	100

Chart 3 : Age wise distribution of carcinoma of cervix

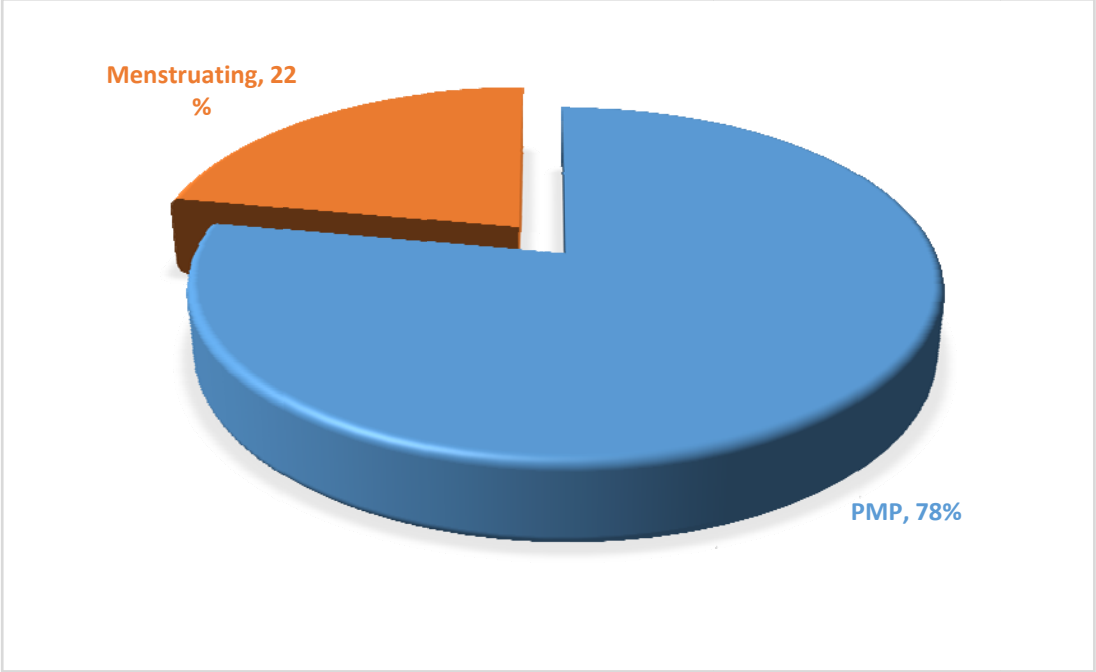


In our study group, carcinoma of cervix was found most commonly in post menopausal females (78%). The incidence in menstruating females were 22%.

Table 4: **Incidence of carcinoma with respect to menstrual status**

Menstrual status	No. of cases	Percentage
Post menopausal	39	78
Menstruating	11	22
Total	50	100

Chart 4 :Incidence of carcinoma with respect to menstrual status

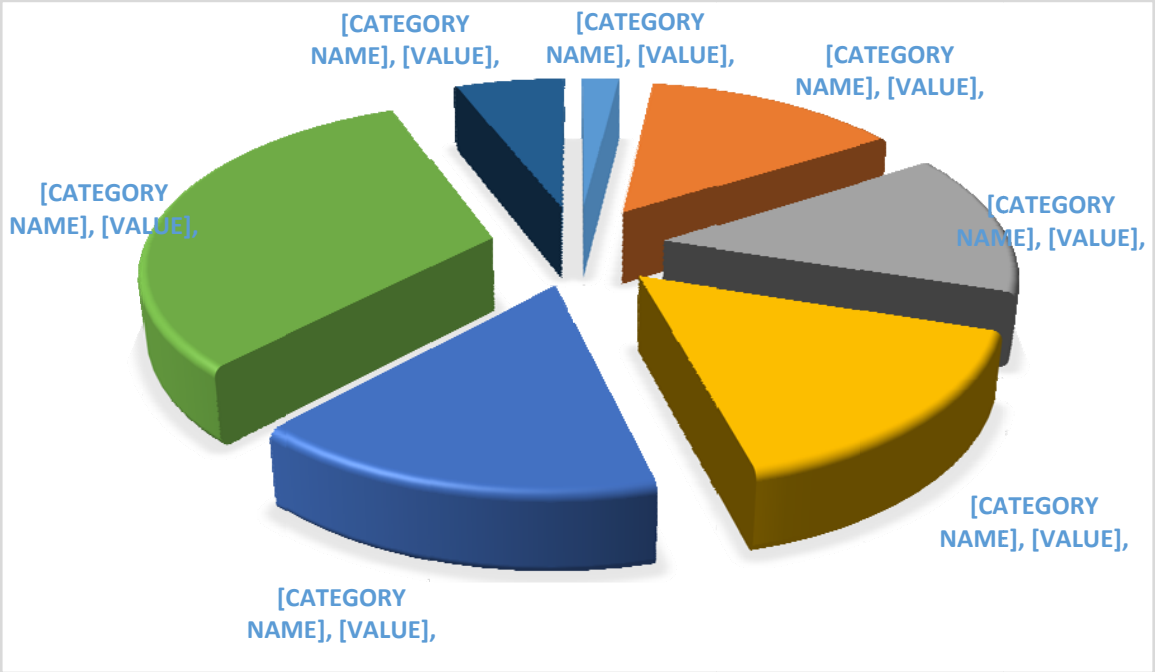


Of the 50 cases included in our study, most common stage was stage IIIB which comprised 16 cases (32%) followed by stage IIB and stage IIIA each of which comprising 8 cases (16%) each.

Table 5 : Incidence of carcinoma with respect to stage of the disease

Stage	No. of cases	Percent
IA	1	2.0
IB	7	14.0
II A	7	14.0
IIB	8	16.0
III A	8	16.0
III B	16	32.0
IV	3	6.0
Total	50	100.0

Chart 5 :: Incidence of carcinoma with respect to stage of the disease

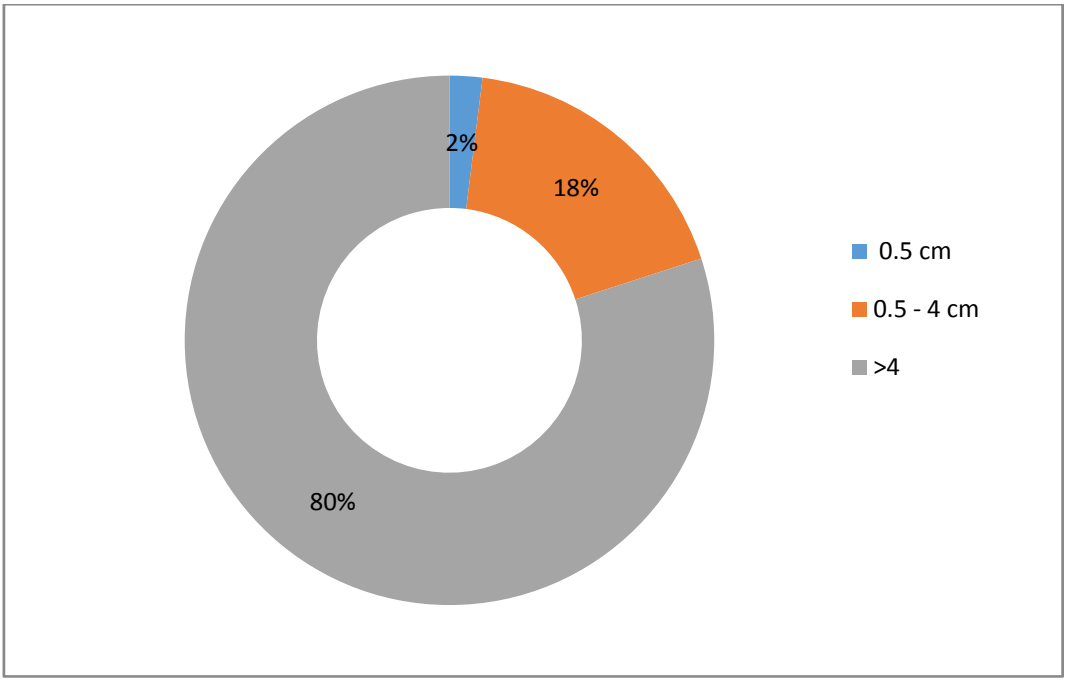


In this study, the size of tumor ranged from less than 0.5 cm to 8cm. The tumor size was categorised as less than 0.5cm, 0.5 – 4 cm and > 4 cm in accordance with the FIGO staging criteria. Among 50 cases most of the tumors were more than 4 cm in size.

Table 6 : Incidence of carcinoma with respect to tumor size

Tumor size	Frequency	Percentage
<0.5 cm	1	2.0
0.5 – 4 cm	9	18.0
>4 cm	40	80.0
Total	50	100.0

Chart 6 : Incidence of carcinoma with respect to tumor size

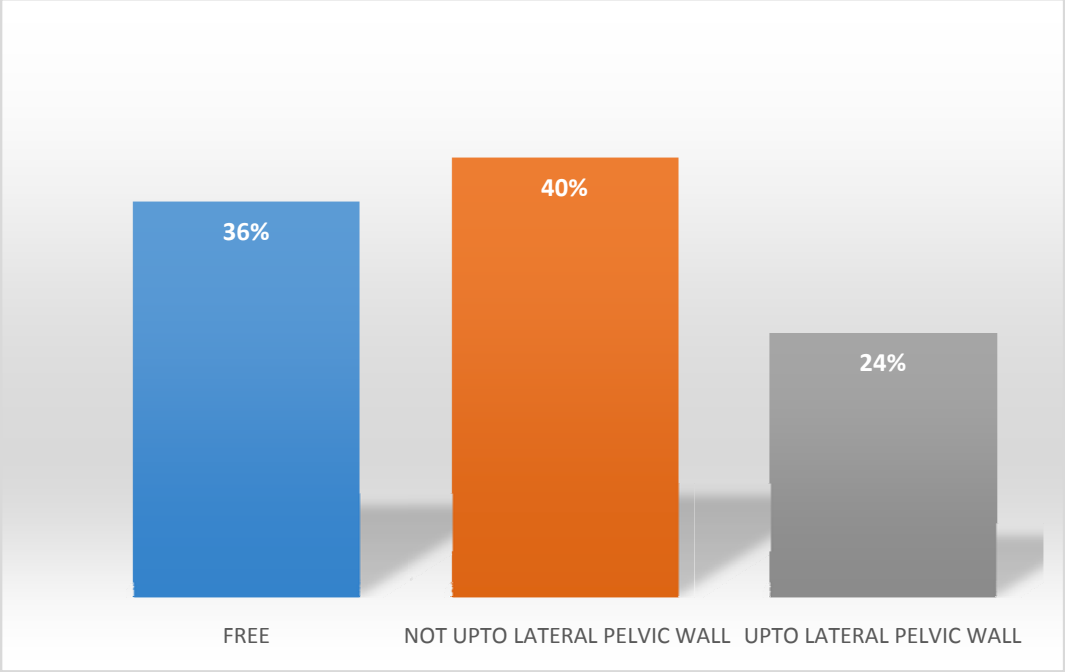


Since majority of cases included in the study were of higher stages, (stage IIIB & stage IIIA), 64% of them had parametrial involvement, 24% upto the lateral pelvic wall. 36% of cases had no parametrial involvement.

Table 7: Incidence of parametrial involvement

Parametrial involvement	No. of cases	Percentage
Parametrium free	18	36
Parametrial invasion not upto lateral pelvic wall	20	40
Parametrial invasion upto lateral pelvic wall	12	24
Total	50	100

Chart 7 : Incidence of parametrial involvement

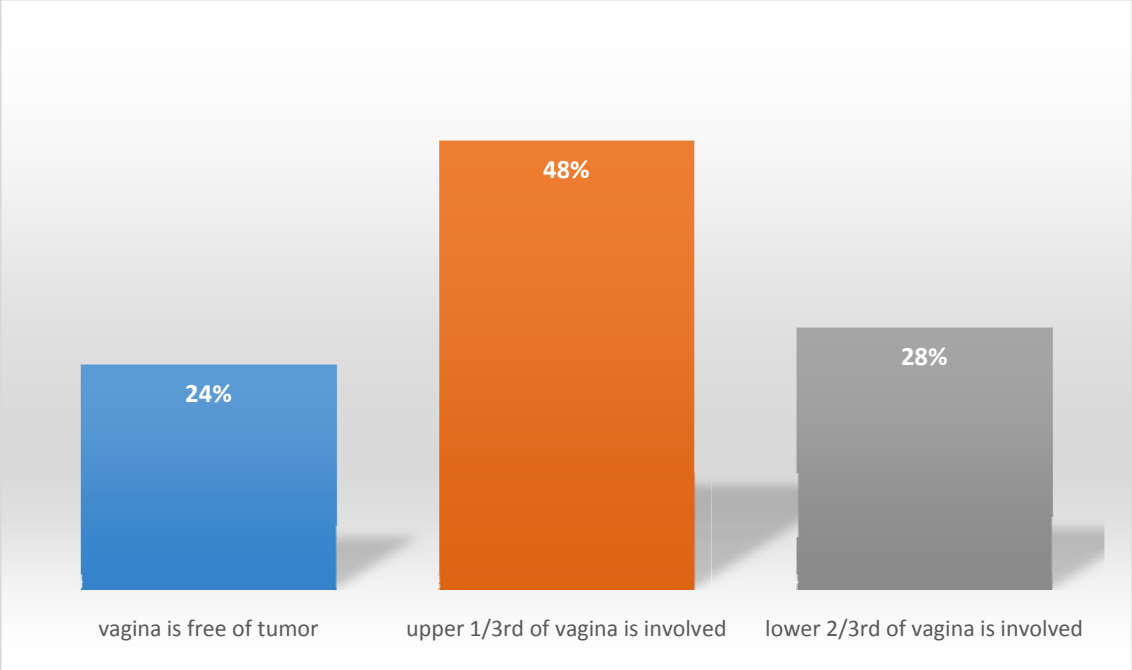


Similar to parametrial involvement, vaginal involvement was also present in majority of cases (76%). In 48% of cases only upper 1/3rd of vagina was involved, whereas in 28% of cases vagina was involved upto lower 1/3rd. Vagina was free of tumor infiltration in 24% of cases.

Table 8: Incidence of vaginal involvement

Vaginal involvement	No. of cases	Percentage
Vagina is free of tumor	12	24
Upper 1/3rd of vagina is involved	24	48
Lower 2/3rd of vagina is involved	14	28
Total	50	100

Chart 8 :Incidence of vaginal involvement

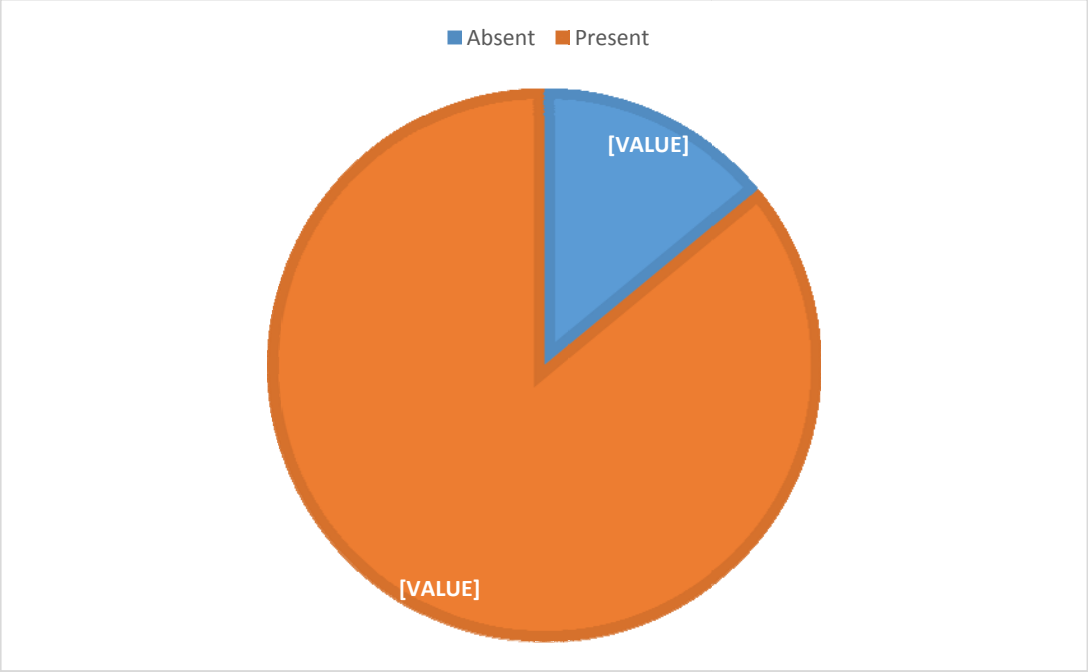


86% of the cases under study had enlarged lymph nodes which were detected radiologically, whereas in 14% of cases nodes were not detected.

Table 9 : Nodal status of the cases

Nodal status	No. of cases	Percentage
Nodes present	43	86
Nodes absent	7	14
Total	50	100

Chart 9 : nodal status of the cases



Among the 50 cases of carcinoma of cervix included in our study, 41 were small biopsies and 9 were hysterectomies. All the 9 hysterectomy cases received in our department during our study period were included in our study, as they satisfied the inclusion criteria for the study.

Table10 : Types of specimen

Type of specimen	No. of cases	Percentage
Cervix biopsy	41	82
Hysterectomy	9	18
Total	50	100

Chart 10 : : Types of specimen

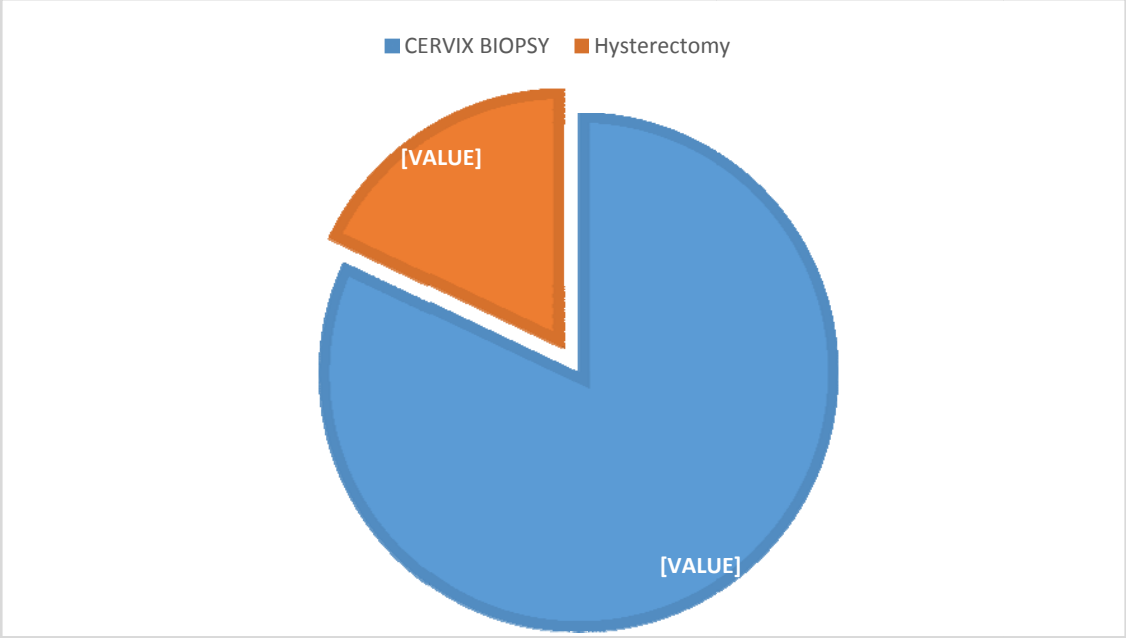
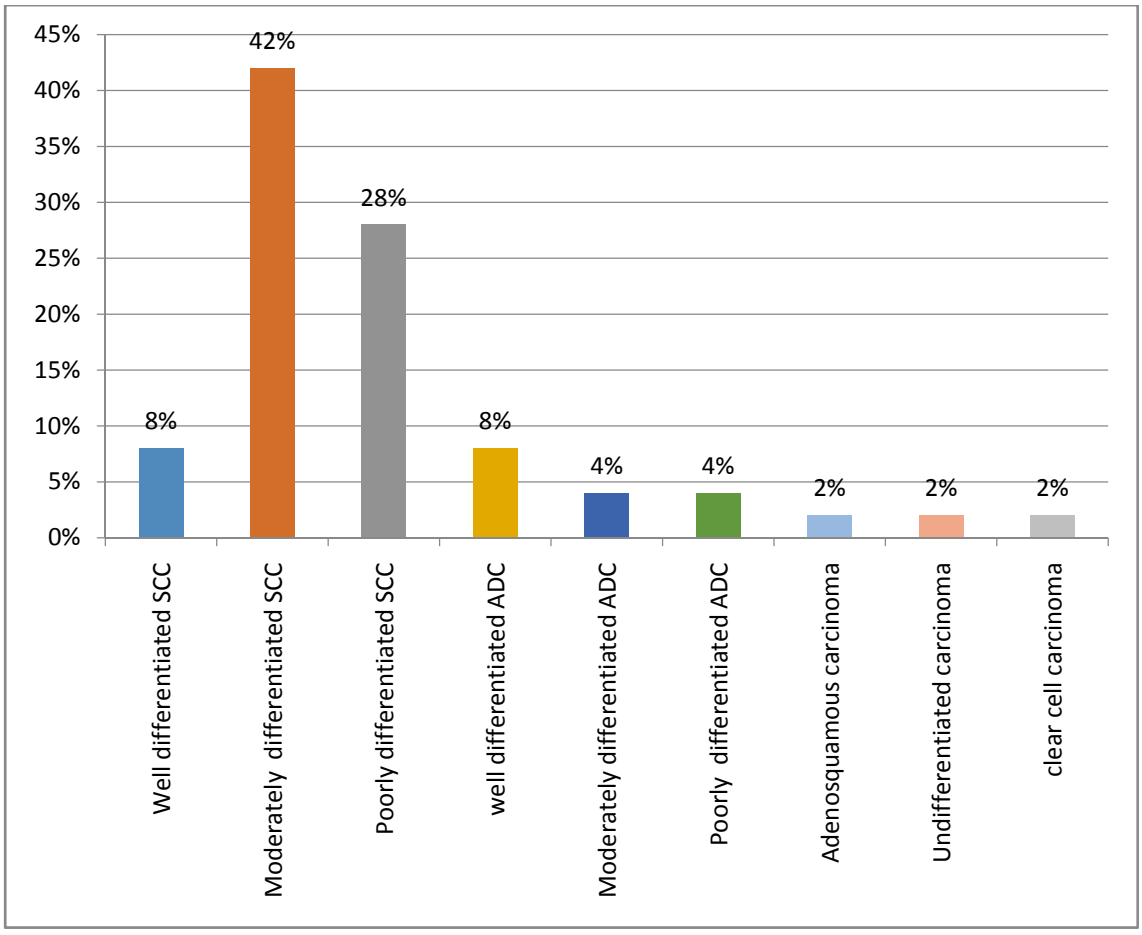


Table 11 : Histological subtypes of cervical carcinoma

Histological subtypes	No. of cases	Percentage
Well differentiated SCC	4	8
Moderately differentiated SCC	21	42
Poorly differentiated SCC	14	28
Well differentiated Adenocarcinoma	4	8
Moderately differentiated Adenocarcinoma	2	4
Poorly differentiated Adenocarcinoma	2	4
Adenosquamous carcinoma	1	2
Undifferentiated carcinoma	1	2
Clear cell carcinoma	1	2
Total	50	100

Of the total 511 cases of cervical carcinoma, squamous cell carcinoma was the most common type, followed by adenocarcinoma. In our study group of 50 cases also squamous cell carcinoma was the most common type. Among squamous cell carcinomas, most common was moderately differentiated grade (54%), followed by poorly differentiated grade (36%).

Chart 11 : Histological subtypes of cervical carcinoma

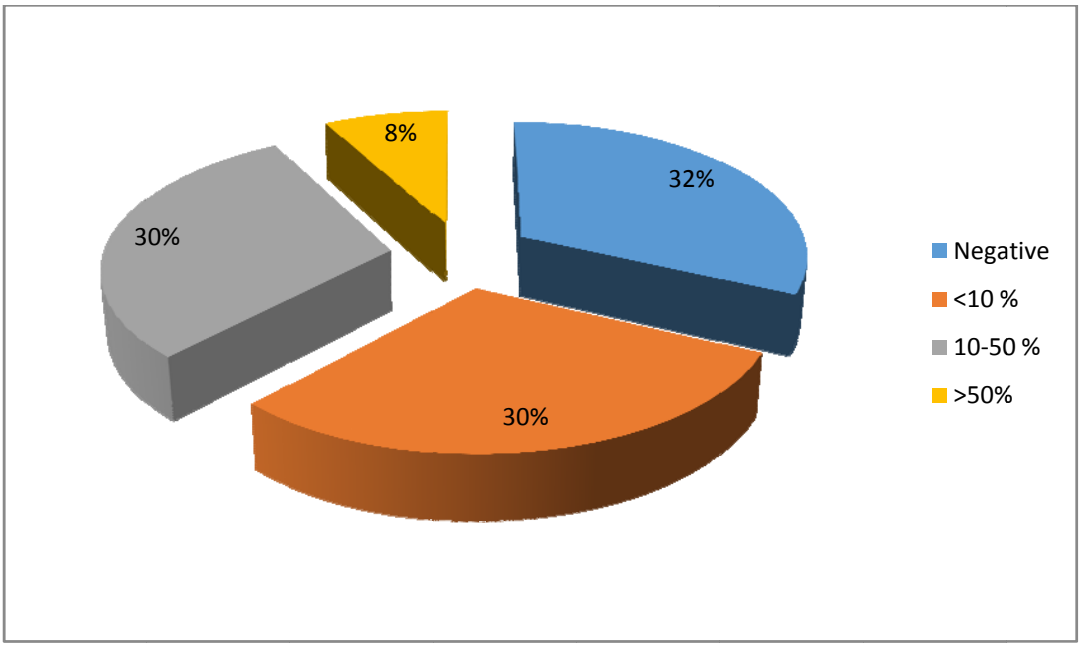


COX2 expression was graded as negative when no tumor cells showed positive staining, 1+ when <10% of tumor cells showed positive staining, 2+ when 10% – 50% of tumor cells showed positive staining and 3+ when more than 50% of tumor cells showed positive staining.

Table 12: COX2 expression in the study group

COX2 expression	No. of cases	Percentage
Negative	16	32
<10%	15	30
10% - 50%	15	30
>50%	4	8
Total	50	100

Chart 12 : COX2 expression in the study group



In the study group, a correlation between age of the patients and histopathological diagnosis was done. It was found that in the age group of 40-50 years, most common was squamous cell carcinoma moderately differentiated grade, whereas in older age groups (51 – 70 years) squamous cell carcinoma poorly differentiated grade was found to be more common. In the case of adenocarcinomas, well differentiated grade was found to be more common in 40-50 years age group whereas poorly differentiated grade was more common in older age group (51-70 years). But this was not statistically significant. Adenosquamous carcinoma and undifferentiated carcinoma was also seen in older age group (51- 60 years) and clear cell carcinoma of cervix was detected in a younger female (40-50 year). Since only a single case was present for these variants these are also statistically not significant.

Table 13 : Correlation between age and HPE subtypes

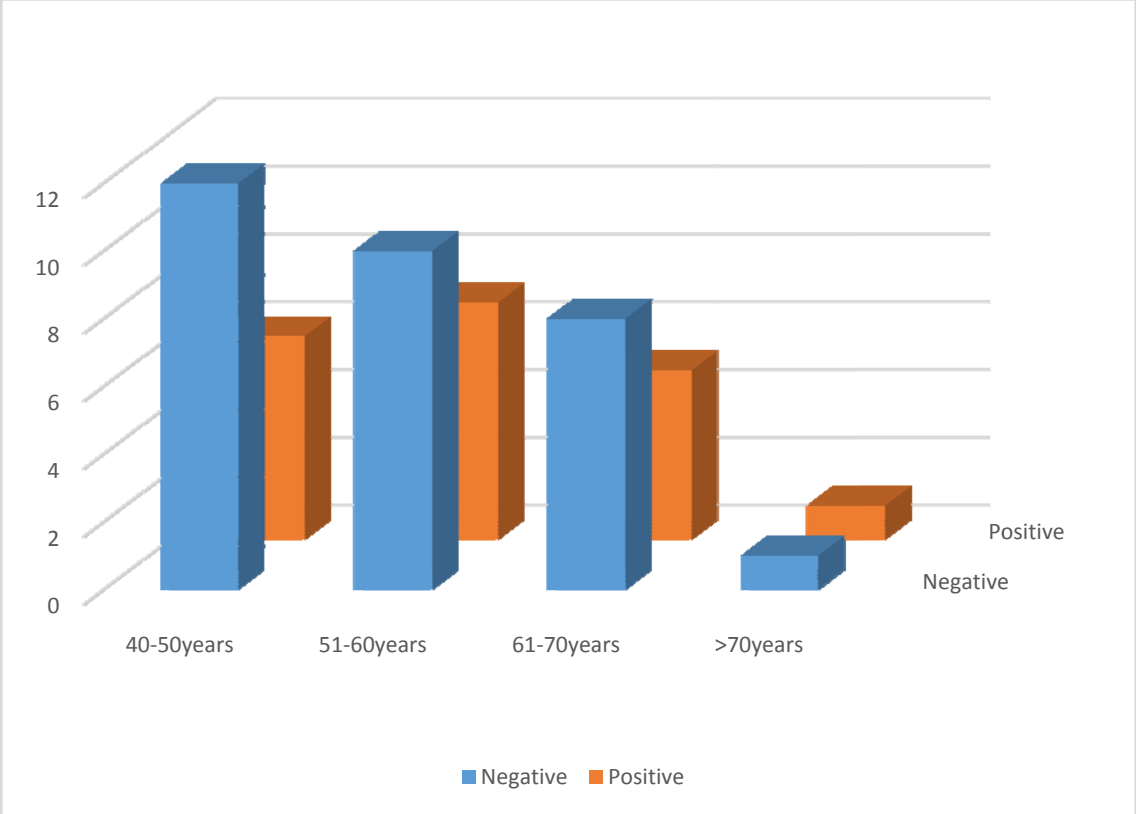
HPE subtypes	Age groups				
	40-50	**51-60**	**61-70**	**>70**	**Total**
Well differentiated SCC	0	2	2	0	4
Moderately differentiated SCC	11	6	3	1	21
Poorly differentiated SCC	2	7	4	1	14
Well differentiated ADC	3	1	0	0	4
Moderately differentiated ADC	1	0	1	0	2
Poorly differentiated ADC	0	1	1	0	2
Adenosquamous carcinoma	0	0	1	0	1
Undifferentiated carcinoma	0	0	1	0	1
Clear cell carcinoma	1	0	0	0	1
Total	18	17	13	2	50

When age of the patients was correlated with COX2 expression, it was found that the expression of COX2 was lesser in younger females i.e 40-50 years age group (33.33%) when compared to females of older age group i.e age more than 50 years (40. 26%). This was also statistically not significant.

Table 14: Correlation between age and COX2 expression

COX2 expression	Age group				
	40-50	**51-60**	**61-70**	**>70**	**Total**
Negative	12	10	8	1	31
Positive	6	7	5	1	19
Total	18	17	13	2	50

Chart 13 : Correlation between age and COX2 expression

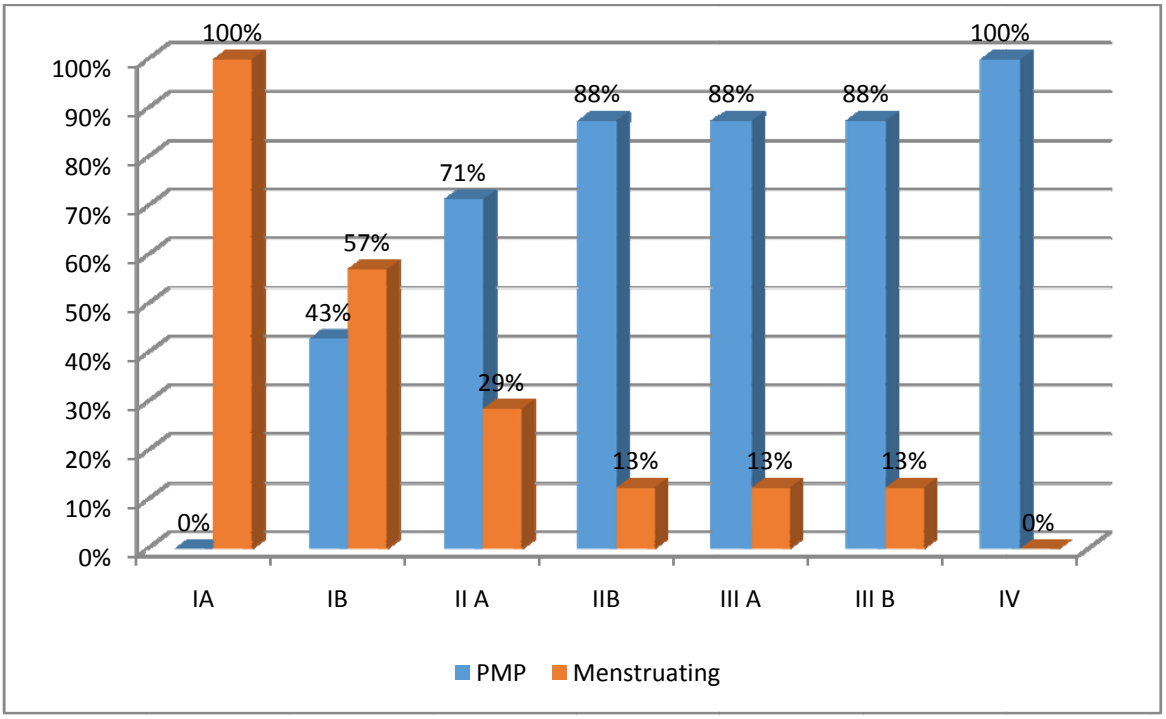


When the menstrual status and stage of the disease were compared, it was noted that menstruating females presented in early stages of the disease, whereas postmenopausal females presented in advanced stages of disease. This correlation was statistically found to be significant with a p value of 0.004. This indicated that younger age group females presented in the early stages of disease when compared to older age group females. This can be due to the positive impact of cervical carcinoma screening procedures and increased awareness of carcinoma of cervix among the general population.

Table 15:Correlation between menstrual status and stage

Menstrual status	**Stage**							**Total**
	IA	**IB**	**IIA**	**IIB**	**IIIA**	**IIIB**	**IV**	
Post - menopausal	0 (0%)	3 (42.9 %)	5 (71.4 %)	7 (87.5 %)	7 (87.5 %)	14 (87.5 %)	3 (100 %)	39 (78 %)
Menstruating	1 (100 %)	4 (52.7 %)	2 (28.6 %)	1 (12.5 %)	1 (12.5 %)	2 (12.5 %)	0 (0%)	11 (22 %)
Total	1 (100 %)	7 (100 %)	7 (100 %)	8 (100 %)	8 (100 %)	16 (100 %)	3 (100 %)	50 (100 %)

Chart 14 : Correlation between menstrual status and stage

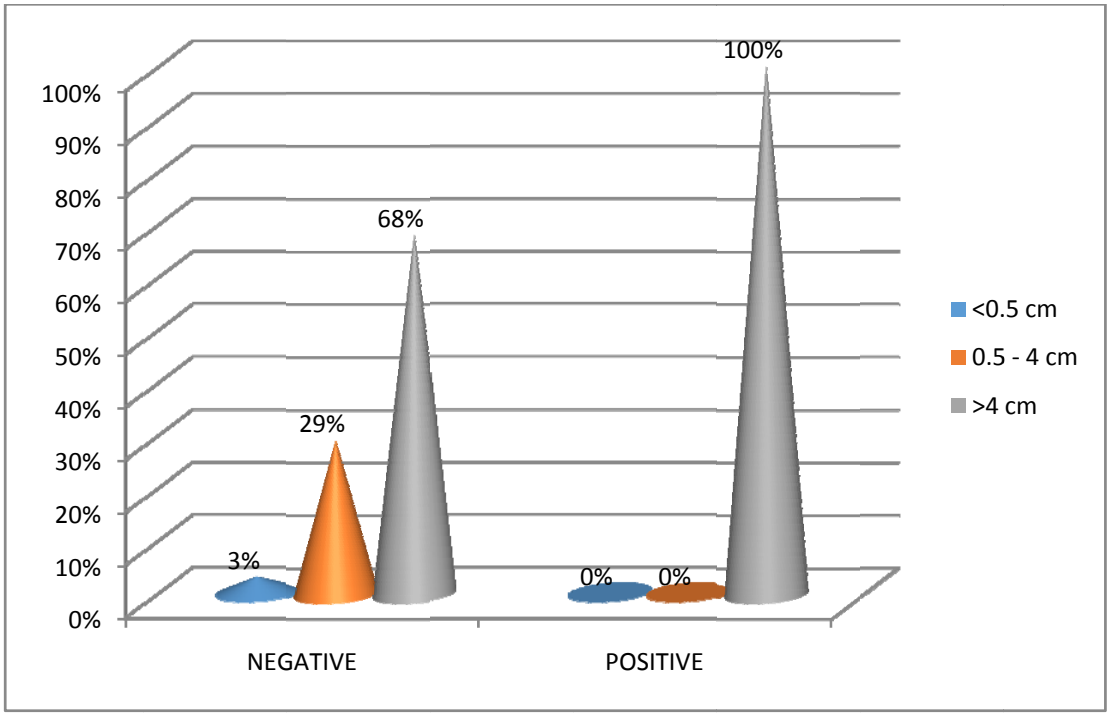


In our study, when the size of the tumors were compared with COX2 expression, it was noted that smaller tumors showed less COX2 expression whereas it was significantly higher for larger tumors. None of the tumors of size less than 4 cm showed positivity for COX2, and in tumors larger than 4cm COX2 expression was 47.5% (19 out of 40 cases). This was found to be statistically significant with a p value of 0.007. This suggests that similar to tumor size, COX2 expression can also be considered as a poor prognostic marker for carcinoma of cervix.

Table 16 : Correlation between tumor size and COX2 expression

Tumor size	COX2 expression		Total
	Negative	Positive	
<0.5 cm	1 (3.2%)	0 (0%)	1 (2%)
0.5 – 4 cm	9 (29%)	0 (0%)	9 (18%)
>4 cm	21 (67.7%)	19 (100%)	40 (80%)
Total	31 (100%)	19 (100%)	50 (100%)

Chart 15 : Correlation between tumor size and COX2 expression

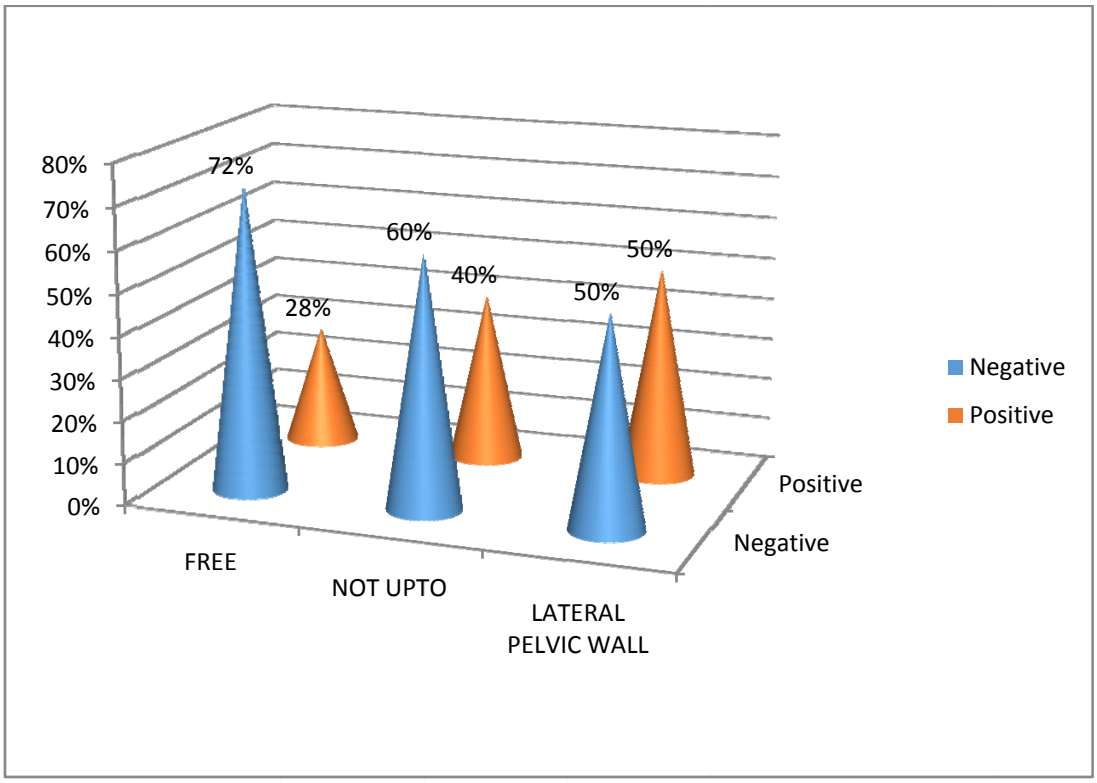


When COX2 expression was compared with the parametrial involvement of the tumor no positive correlation was noted.

Table 17 : Correlation between COX2 expression &parametrial involvement

COX2 expression	Parametrial involvement			Total
	Free	Not upto pelvic wall	Upto pelvic wall	
Negative	13 (72.2%)	12 (60%)	6 (50%)	31 (62%)
Positive	5 (27.8%)	8 (40%)	6 (50%)	19 (38%)
Total	18 (100%)	20 (100%)	12 (100%)	50 (100%)

Chart 16 : Correlation between COX2 expression &parametrial involvement

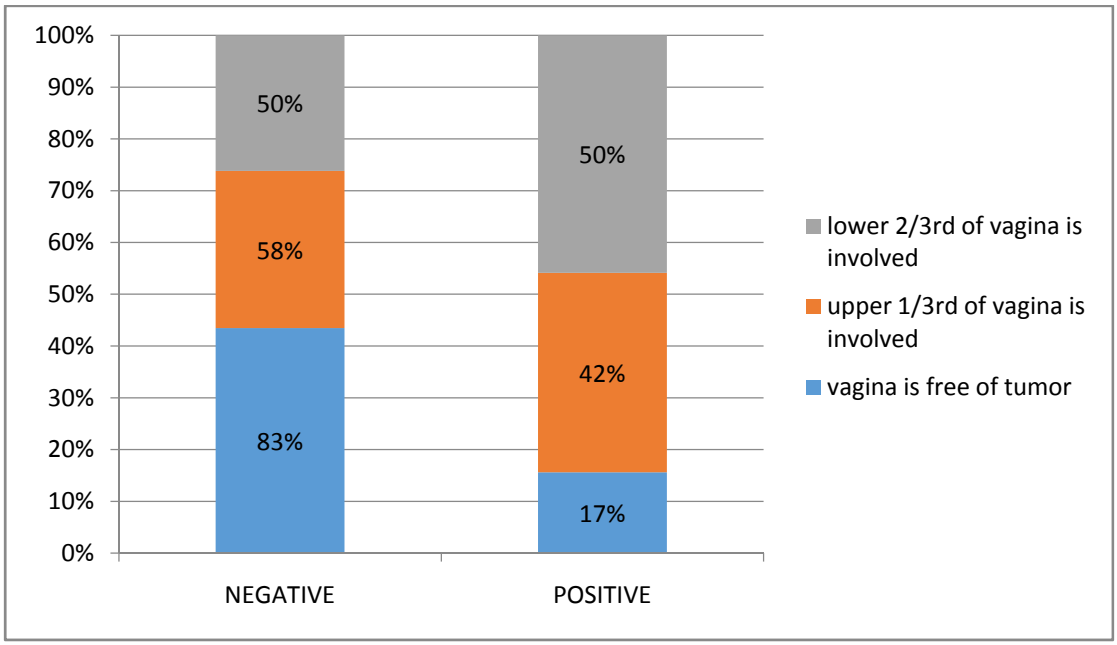


When COX2 expression was correlated with vaginal involvement by the tumor, it was noted that tumors with vaginal involvement were showing increased COX2 expression than those in which vagina was free of tumor.

Table 18 : COX2 expression and vaginal involvement

COX2 expression	Vaginal involvement			Total
	Free	Upper 1/3rd involved	Lower 2/3rd involved	
Negative	10 (83.3%)	14 (58.3%)	7 (50%)	31 (62%)
Positive	2 (16.7%)	10 (41.7%)	7 (50%)	19 (38%)
Total	12 (100%)	24 (100%)	14 (100%)	50 (100%)

Chart 17 : COX2 expression and vaginal involvement

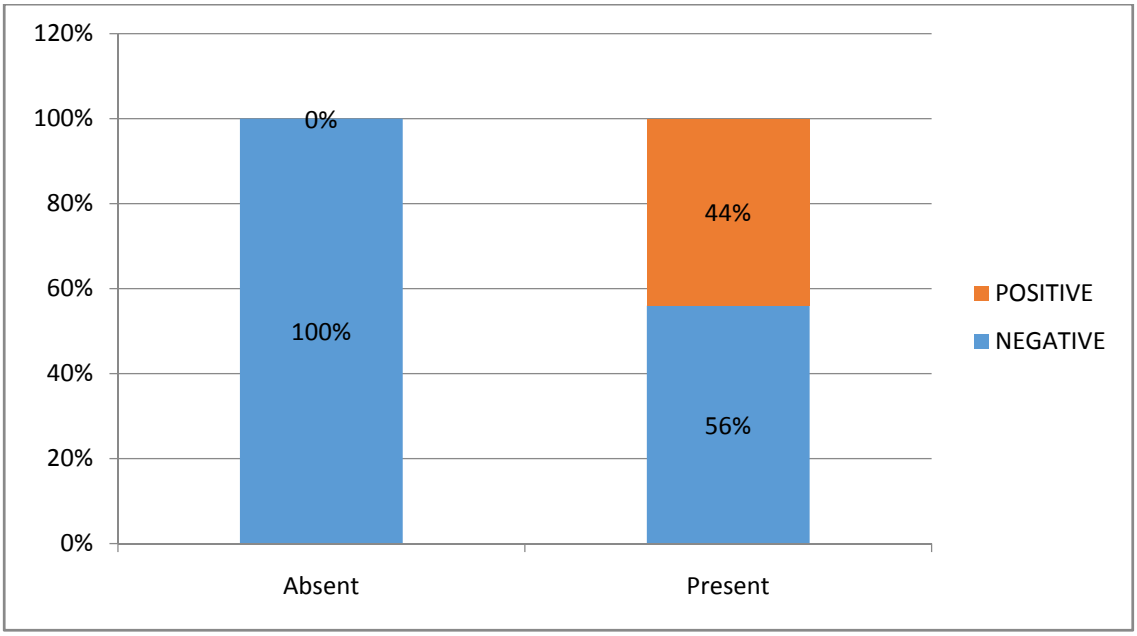


On comparing the COX2 expression with nodal status of the patients, it was noted that in all the 7 cases where no nodes were detected clinically, COX2 expression was negative. Among the 43 cases with enlarged nodes, 44.2% had positive COX2 expression. This was found to be statistically significant with a p value of 0.026.

Table 19: COX2 expression and nodal status

COX2 expression	Nodal status		Total
	Absent	Present	
Negative	7(100%)	24(55.8%)	31(62%)
Positive	0(0%)	19(44.2%)	19(38%)
Total	7(100%)	43(100%)	50(100%)

Chart 18: COX2 expression and nodal status



When COX2 expression was compared with the clinical stage of the disease, it was noted that COX2 expression was more in tumors of higher clinical stage. In lower stages of tumor like stage IA and stage IB, none of the tumors showed COX2 positivity. In higher stages of tumor, COX2 expression was also noted to be more. However, this correlation was not found to be statistically significant.

Table 20: COX2 expression and clinical stage

Clinical stage	**COX2 expression**		**Total**
	Negative	**Positive**	
IA	1	0	1
IB	7	0	7
IIA	4	3	7
IIB	6	2	8
IIIA	3	5	8
IIIB	7	9	16
IV	3	0	3
Total	**31**	**19**	**50**

When COX2 expression was correlated with the histopathological subtype of carcinoma, it was seen that higher COX2 expression was present in squamous cell carcinoma (42%), than in adenocarcinoma (25%).

Table 21: COX2 expression & histological subtypes of carcinoma

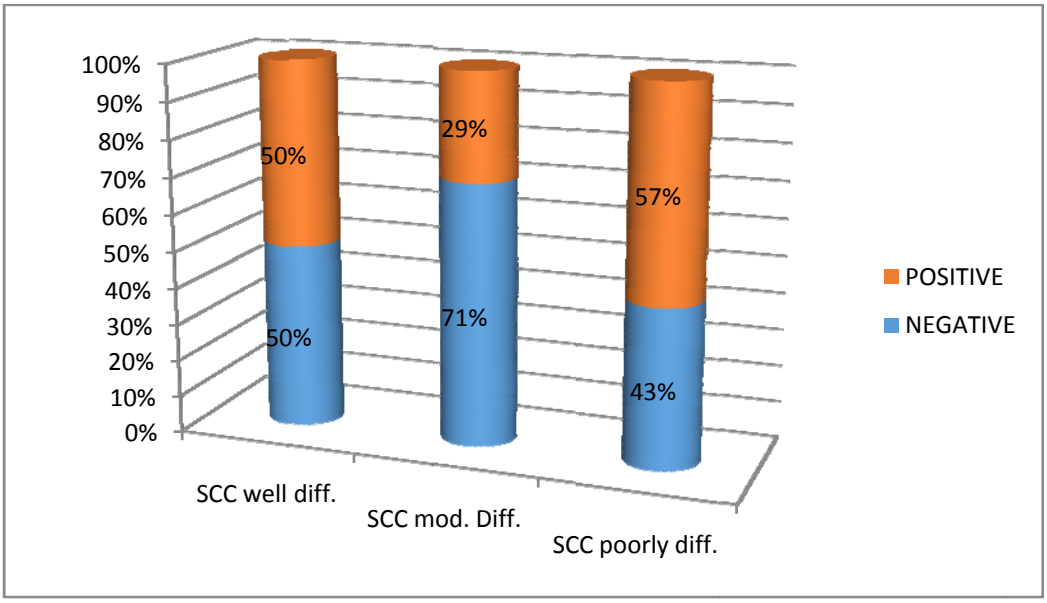
Histological subtypes	COX2 expression		Total
	Negative	Positive	
Well differentiated SCC	2	2	4
Moderately differentiated SCC	15	6	21
Poorly differentiated SCC	6	8	14
Well differentiated ADC	3	1	4
Moderately differentiated ADC	1	1	2
Poorly differentiated ADC	2	0	2
Adenosquamous carcinoma	1	0	1
Undifferentiated carcinoma	1	0	1
Clear cell carcinoma	0	1	1
Total	**31**	**19**	**50**

Among squamous cell carcinoma, COX2 expression was found to be higher in poorly differentiated variant. For well differentiated variant, COX2 positivity was 50%, for moderately differentiated variant 28.6% and for poorly differentiated variant 57.1%.

Table 22 : COX2 expression in SCC variants

SCC variants	COX2 expression		Total
	Negative	Positive	
Well differentiated SCC	2 (50%)	2 (50%)	4 (100%)
Moderately differentiated SCC	15 (71.4%)	6 (28.6%)	21 (100%)
Poorly differentiated SCC	6 (42.9%)	8 (57.1%)	14 (100%)
Total	**23 (59%)**	**16 (41%)**	**39 (100%)**

Chart 19 : COX2 expression in SCC variants

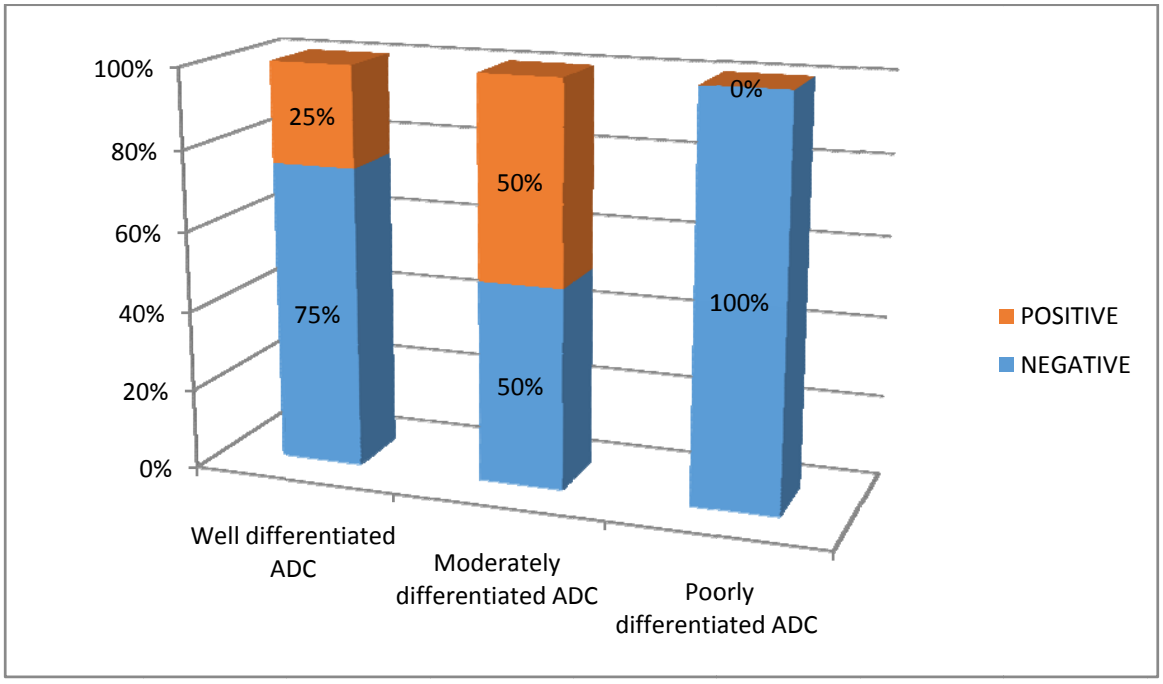


In case of adenocarcinoma, no correlation was noted on comparing the COX2 expression and adenocarcinoma variants.

Table 23 : COX2 expression in adenocarcinoma variants

Adenocarcinoma variants	COX2 expression		Total
	Negative	Positive	
Well differentiated ADC	3 (75%)	1 (25%)	4 (100%)
Moderately differentiated ADC	1 (50%)	1 (50%)	2 (100%)
Poorly differentiated ADC	2 (100%)	0 (0%)	2 (100%)
Total	**6 (75%)**	**2 (25%)**	**8 (100%)**

Chart 20 : COX2 expression in adenocarcinoma variants



GROSS PICTURES OF CARCINOMA OF CERVIX

Figure: 1- Hysterectomy specimen showing a polypoid growth in the cervix

Figure: 2 – Hysterectomy specimen showing growth in cervix

SQUAMOUS CELL CARCINOMA – WELL DIFFERENTIATED

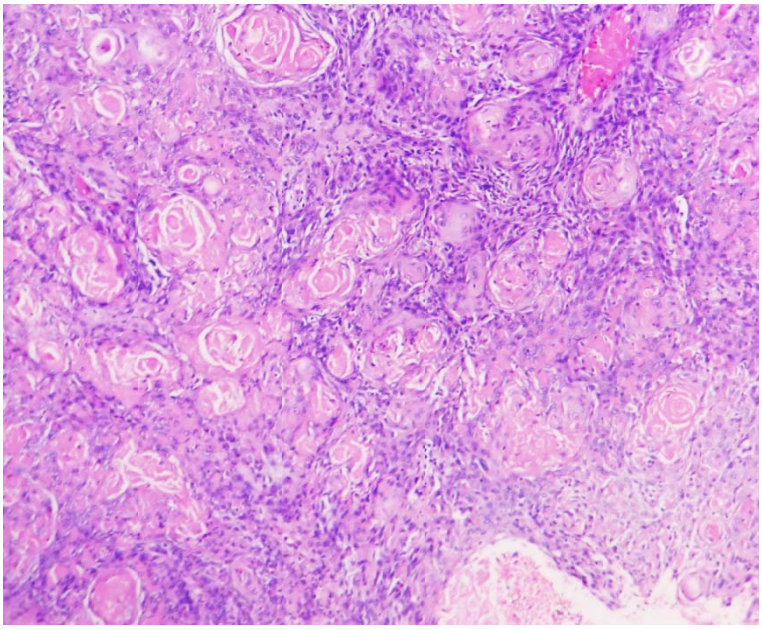


Figure: 3 –Malignant squamous epithelial cells with many keratin pearls H&E100X

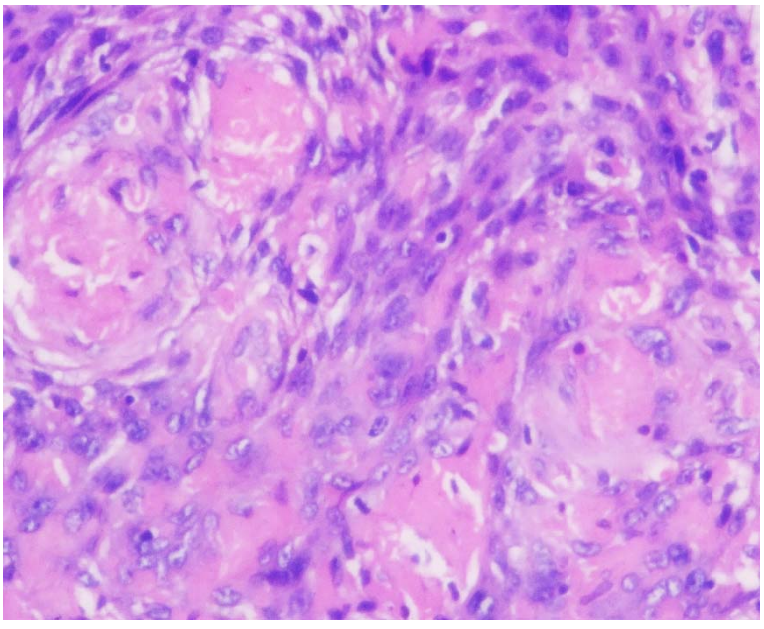


Figure 4-Tumor cells with abundant eosinophilic cytoplasm, pleomorphic hyperchromatic nucleiH&E400X

SQUAMOUS CELL CARCINOMA – MODERATELY DIFFERENTIATED

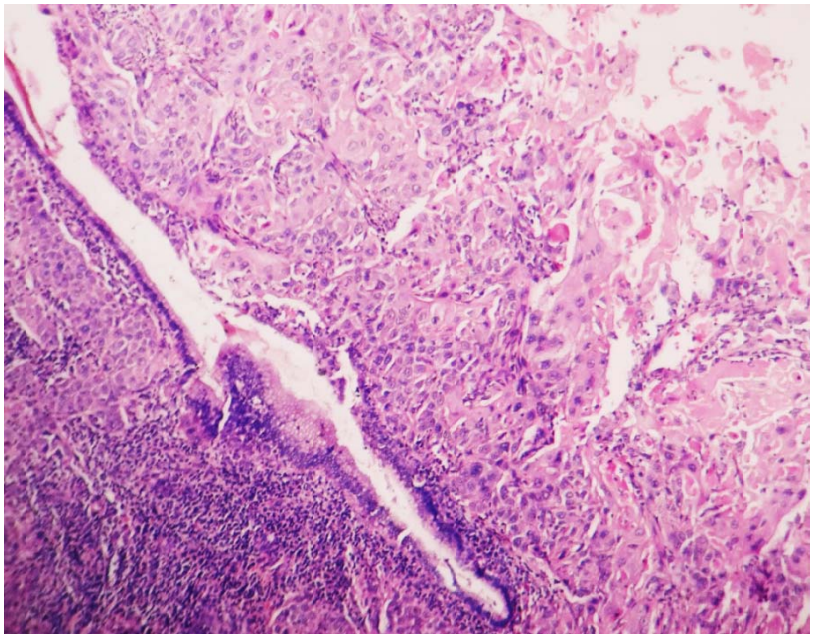


Figure 5 –Endocervical gland with squamous metaplasia that has undergone malignant transformation H&E 100X

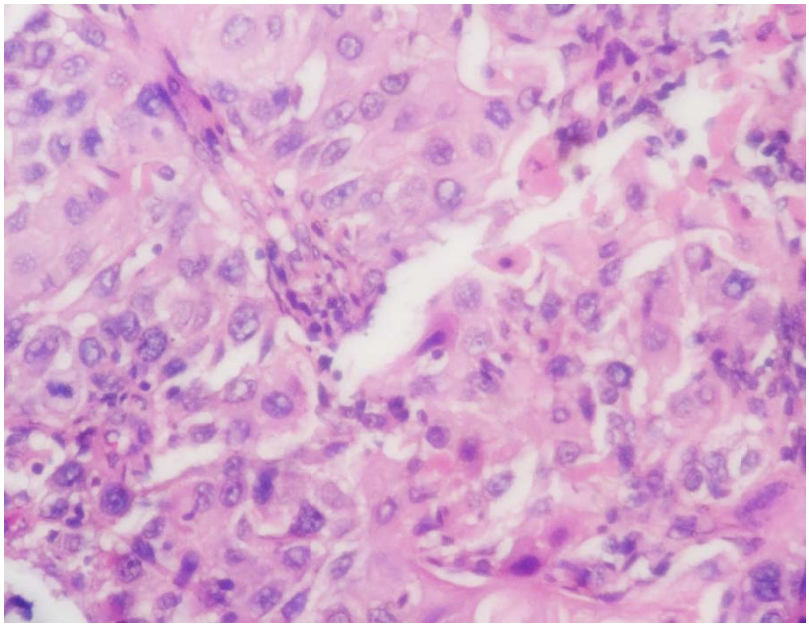


Figure 6- Malignant cells showing pleomorphic nuclei and individual cell keratinization H&E 400X

SQUAMOUS CELL CARCINOMA – POORLY DIFFERENTIATED

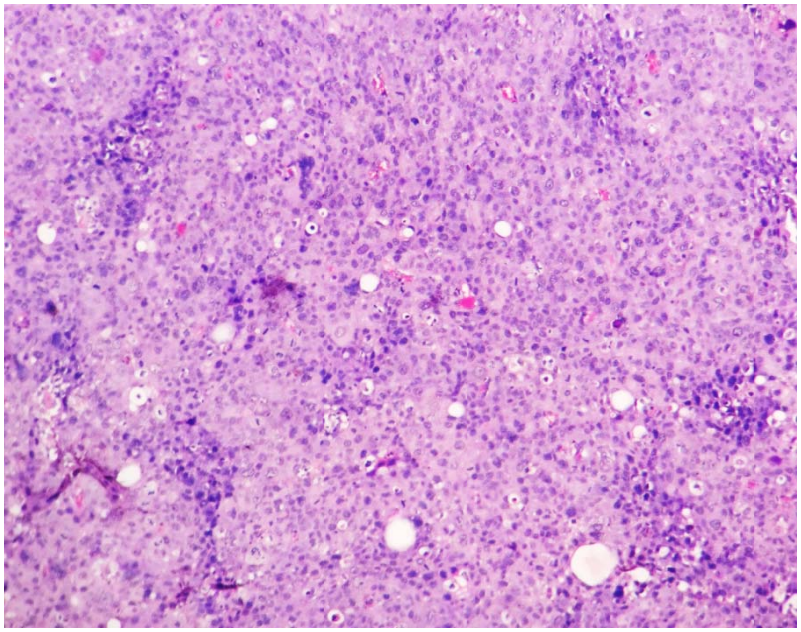


Figure 7: Sheets of tumor cells with no definite evidence of squamous differentiation H&E 100X

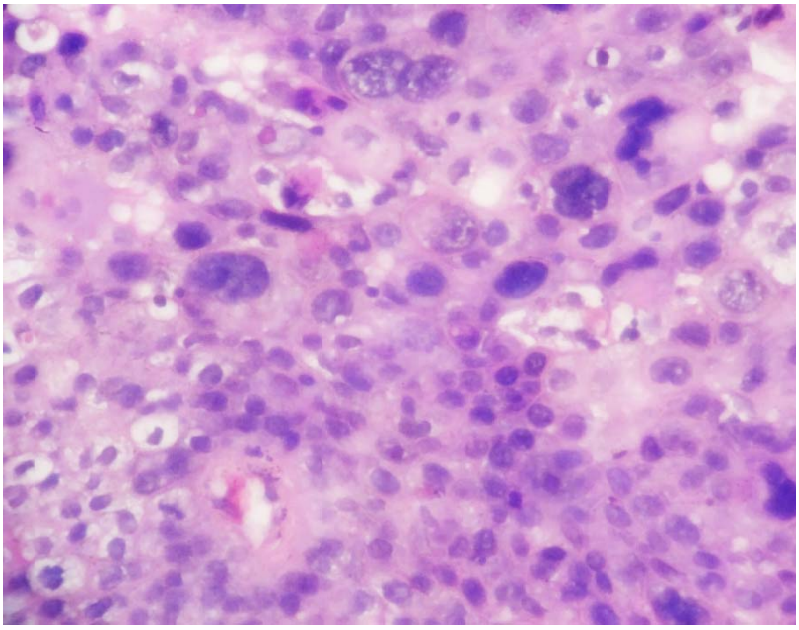


Figure 8 – Tumor cells with with scant indistinct cytoplasm, hyperchromatic oval nuclei &tumor giant cells. H&E 400X

ADENOCARCINOMA - WELL DIFFERENTIATED

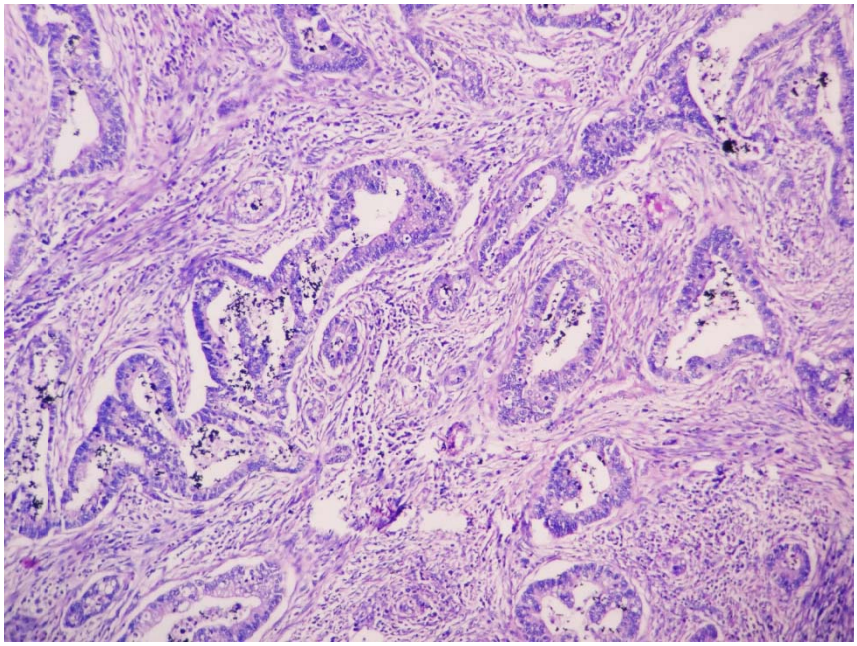


Figure 9 – Malignant glands disposed in a desmoplastic stroma. H&E 100X

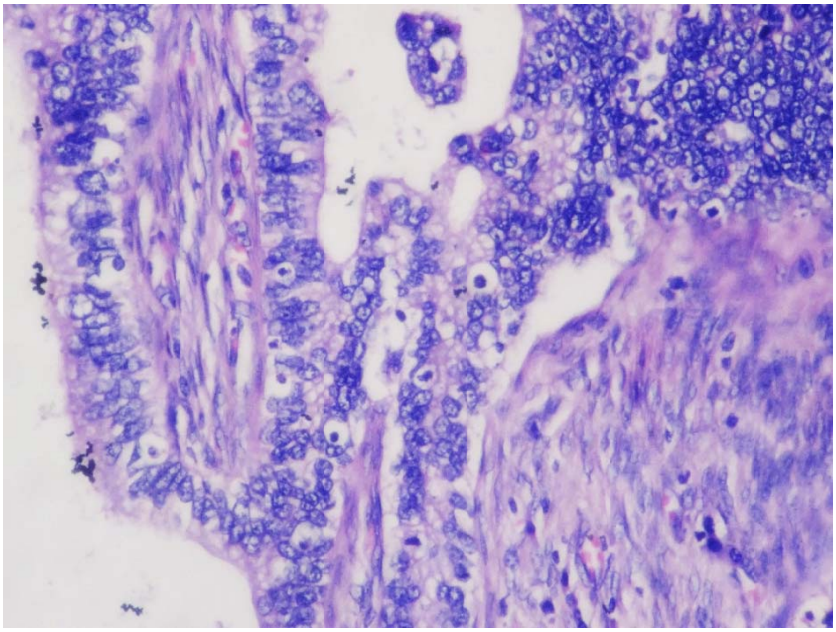


Figure 10 – Malignant glands with stratification, cellular pleomorphism and mitotic figures H&E 400X

ADENOCARINOMA – MODERATELY DIFFERENTIATED

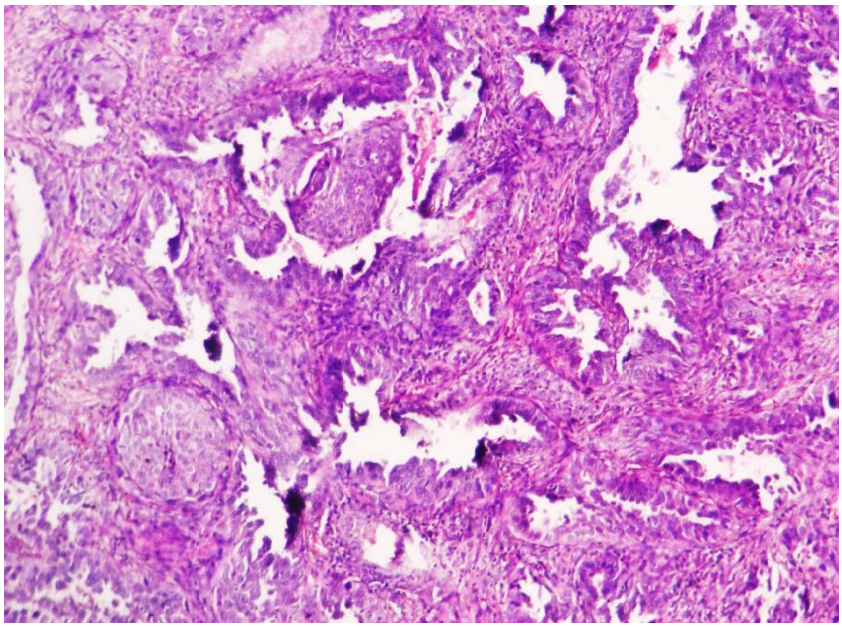


Figure 11 –Malignant glands with <50% of solid areas H&E 100X

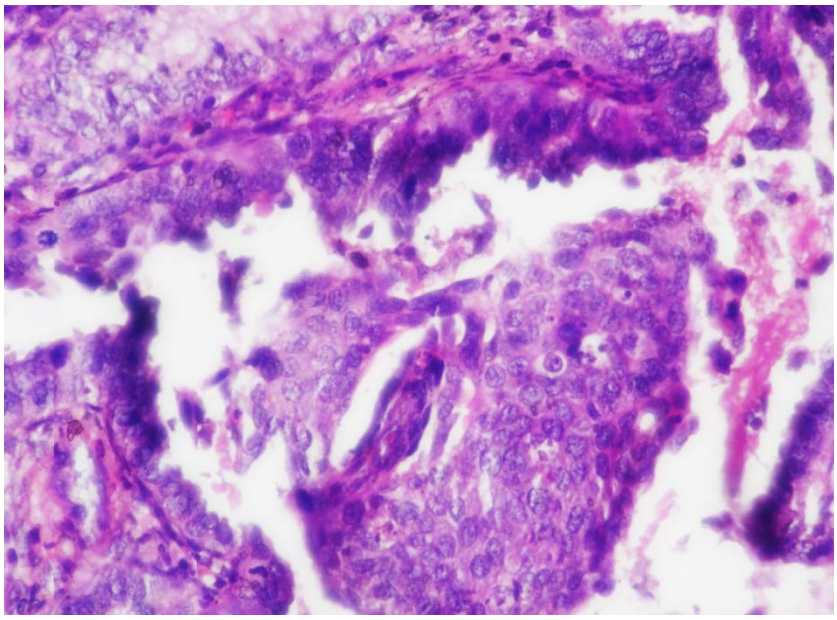


Figure 12 – Malignant cells with moderate eosinophilic cytoplasm pleomorphic vesicular nuclei. H&E 400X

POORLY DIFFERENTIATED ADENOCARCINOMA

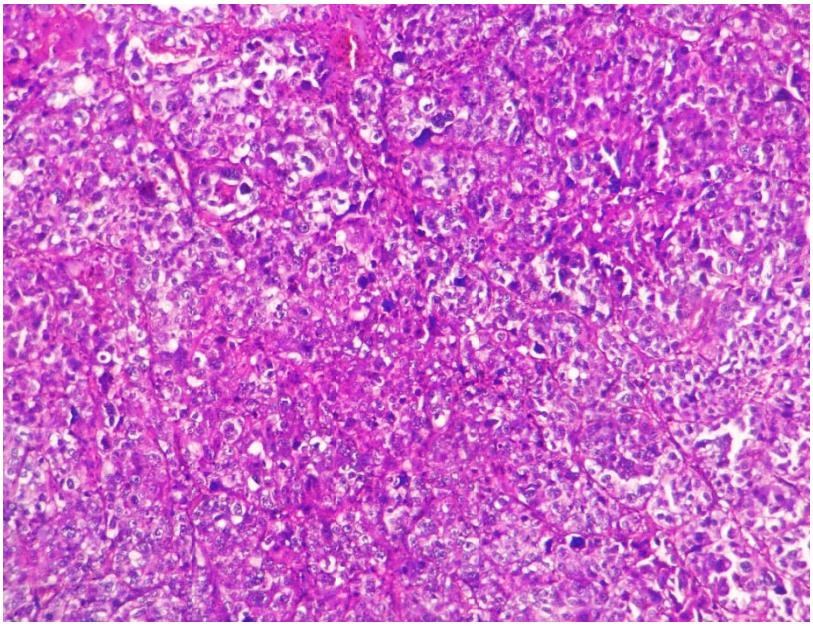


Figure 13 – Sheets of malignant cells with foci showing attempted gland formation. H&E 100X

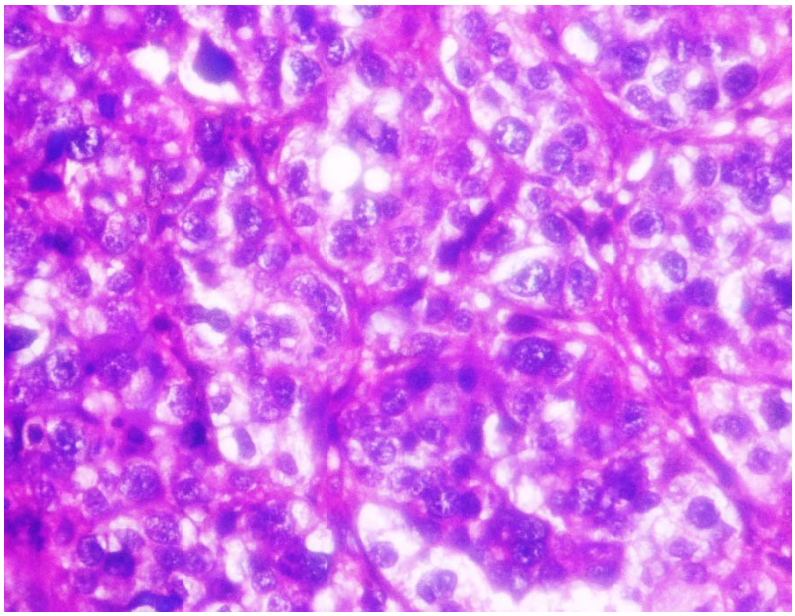


Figure 14 – Malignant cells with clear to eosinophilic cytoplasm, pleomorphic vesicular nuclei, many mitotic figures H&E 400X

ADENOSQUAMOUS CARCINOMA

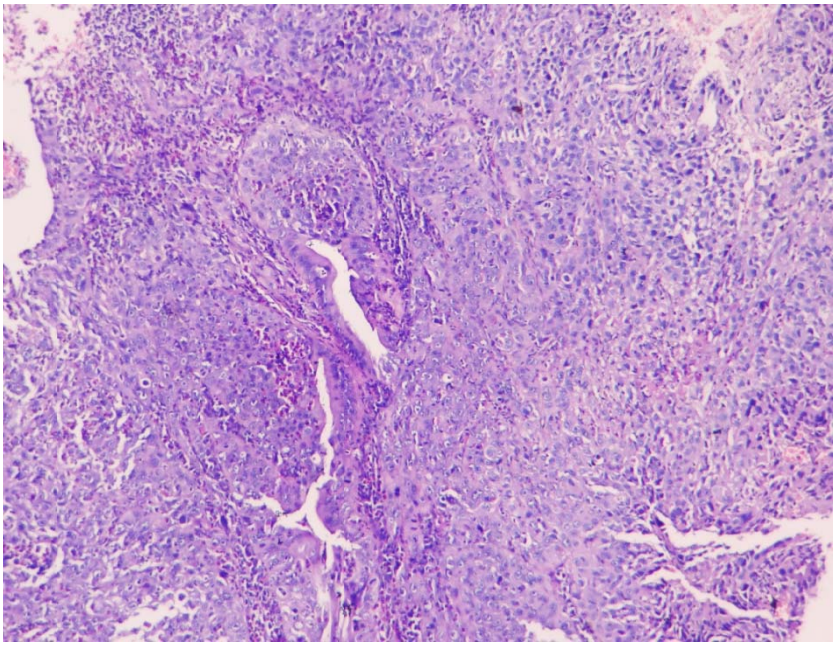


Figure 15 – Shows admixture of malignant gland and sheets of squamous cells. H&E 100X

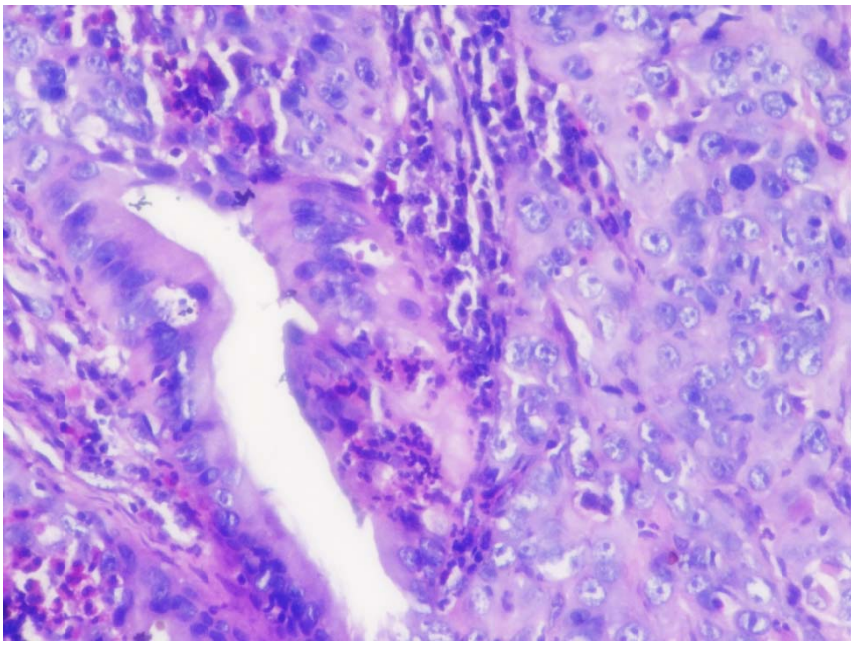


Figure 16 – Malignant glandular epithelial cells with adjacent malignant squamous cells. H&E 400X

CLEAR CELL CARCINOMA OF CERVIX

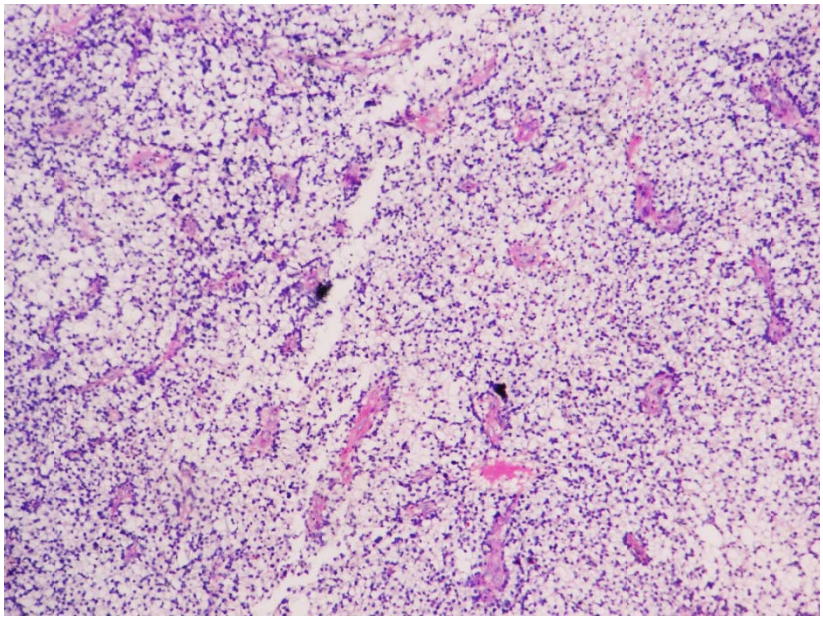


Figure 17 – Sheets of tumor cells with clear cytoplasm. H&E 100X

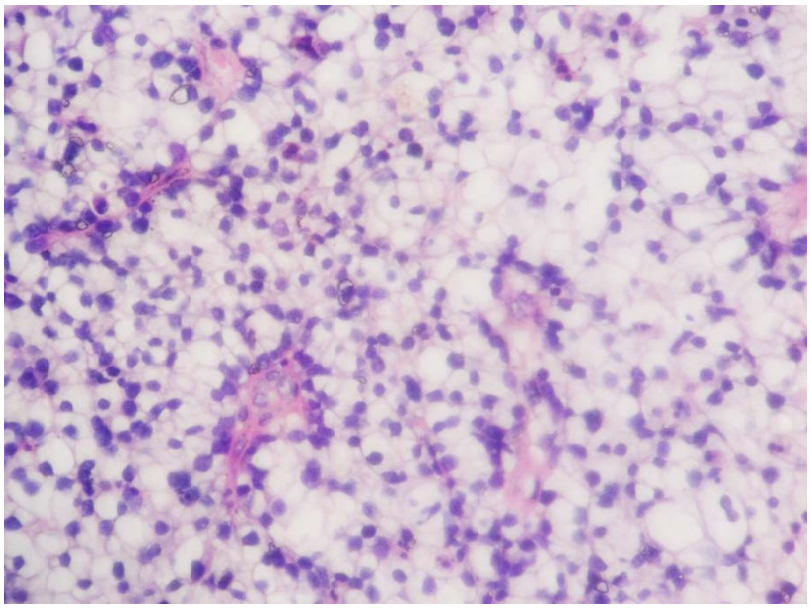


Figure 18 – Tumor cells with well defined cell margins, moderate to abundant clear cytoplasm& high grade nuclei. H&E 400X

UNDIFFERENTIATED CARCINOMA

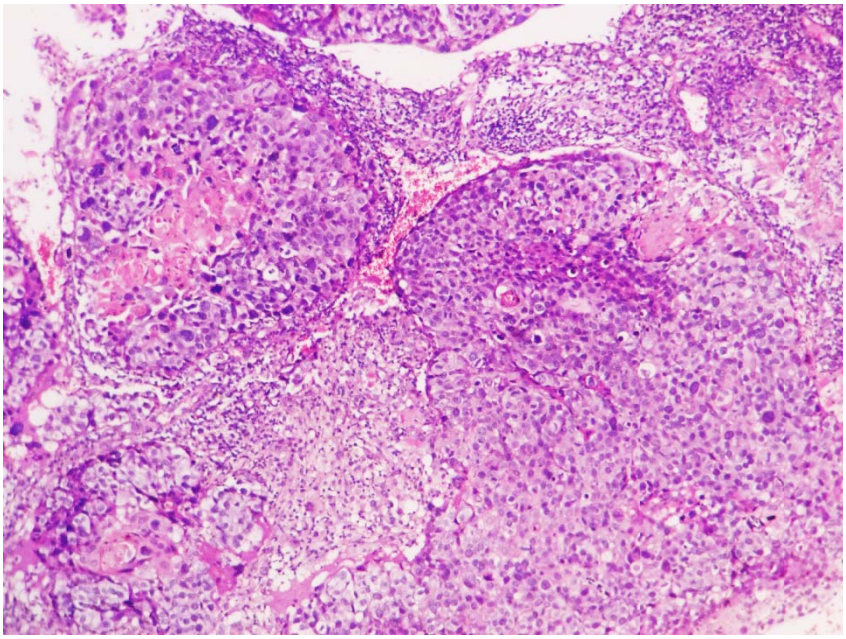


Figure 19 – Malignant cells arranged in sheets and lobules. H&E 100X

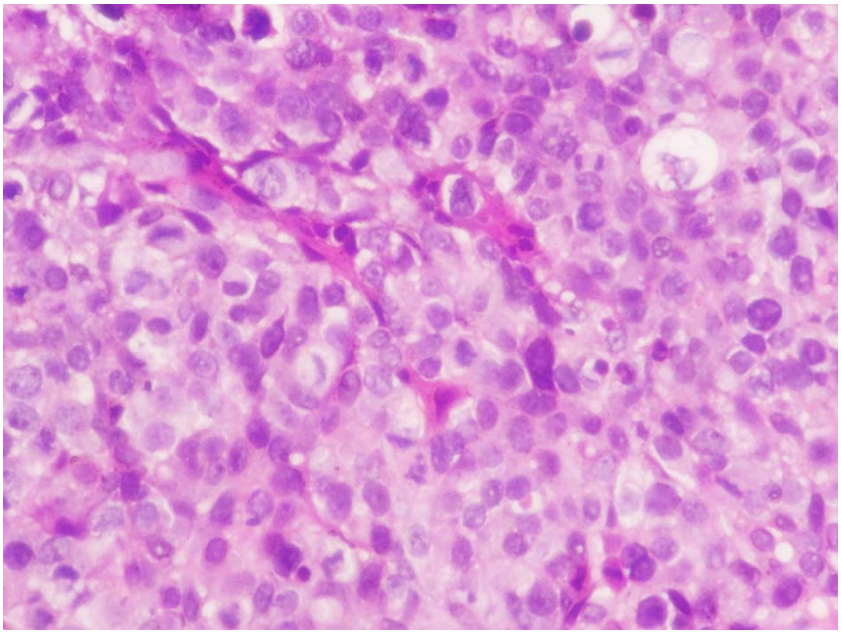


Figure 20- Tumor cells with moderate amount of cytoplasm, pleomorphicnuclei and many mitotic figures. H&E 400X

COX2 EXPRESSION

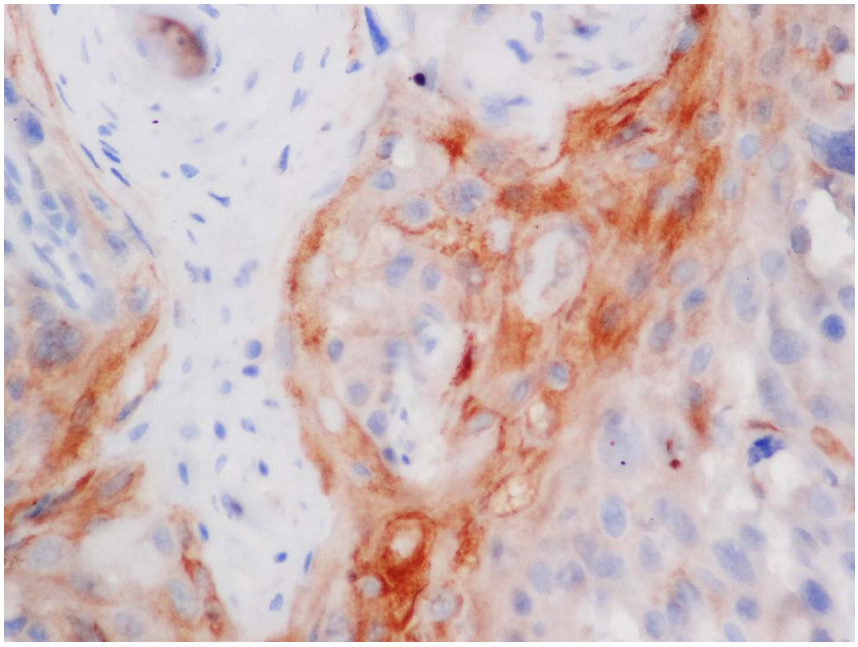


Figure 21 – COX2 cytoplasmic and membrane positivity in well differentiated SCC400X

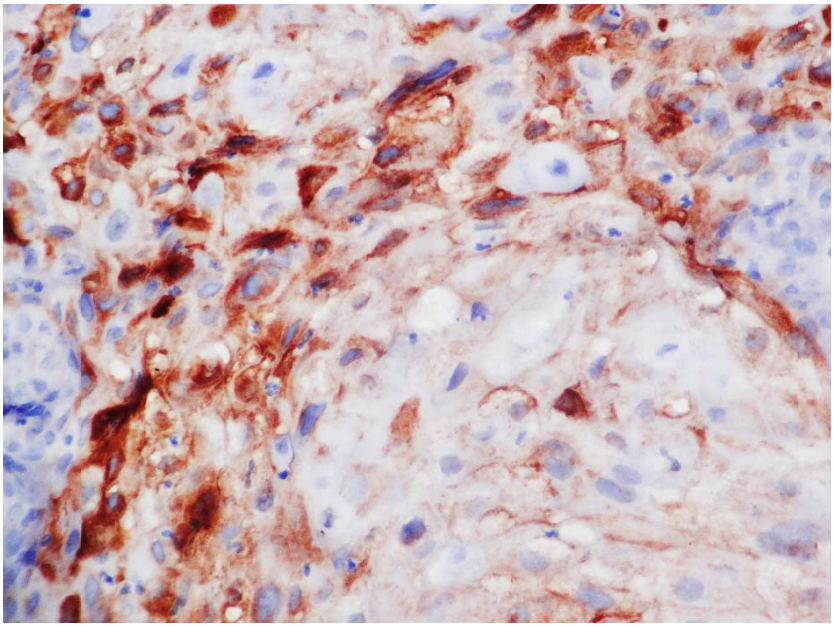


Figure 22 – COX2 positivity in moderately differentiated SCC 400X

COX2 EXPRESSION

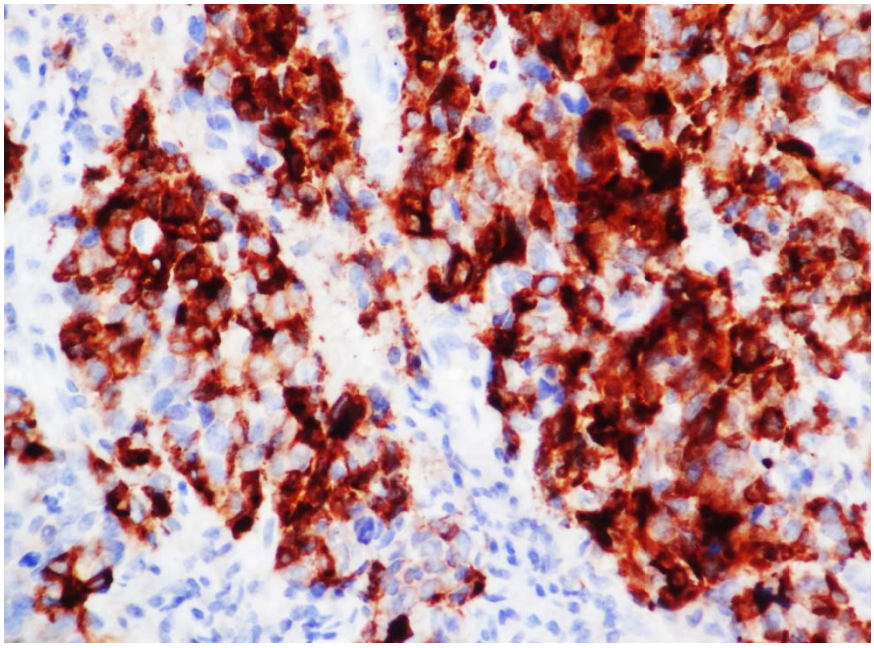


Figure 23 – COX2 positivity in poorly differentiated SCC 400X

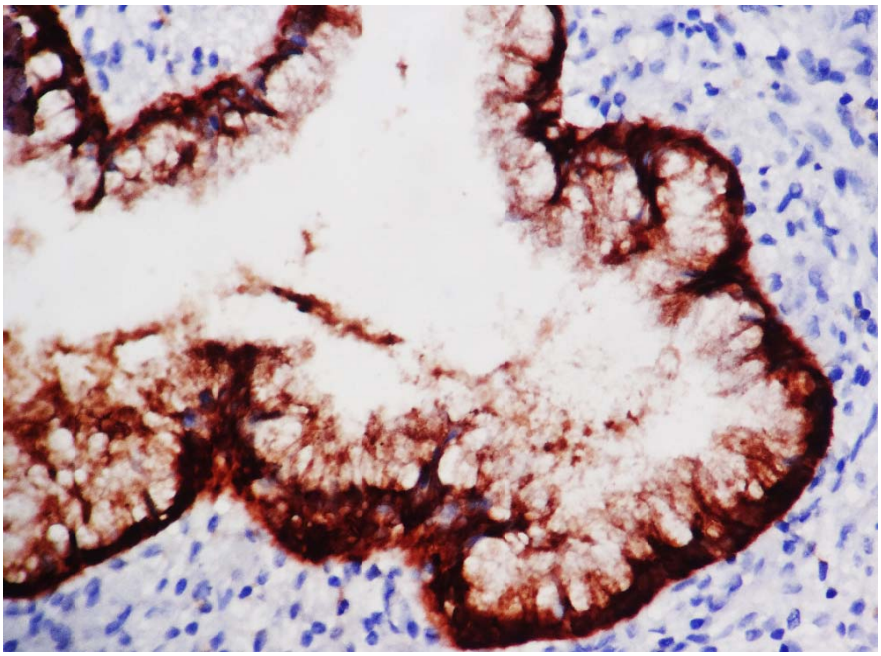


Figure 24 – COX2 positivity in well differentiated Adenocarcinoma 400X

DISCUSSION

DISCUSSION

Cervical carcinoma ranks fourth among the leading causes of cancer worldwide, after carcinoma of breast, colorectal carcinoma and lung carcinoma. In developing countries like India, cervical carcinoma accounts for the second most common cancer occurring in women and also the second leading cancer killer next only to carcinoma breast. According to WHO (2005), more than 5 lakh new cases of carcinoma of cervix are diagnosed every year, of which more than 90% are in developing countries. In India, around 50000 new cases are diagnosed every year.

Cervical carcinoma is unique in that it is one of the most preventable as well as treatable cancer, provided it is detected early in the course. Several screening procedures have been introduced for the early detection of cancer cervix, which includes pap smear, HPV screening, colposcopy, VIA, VILI, cervix biopsy.

The incidence of squamous cell carcinoma of cervix is decreasing for the past two decades. But the incidence of adenocarcinoma is seen to be increasing. According to the National Cancer Registry Programme (ICMR), most of the urban population in India are showing a statistically significant reduction in the incidence of carcinoma of cervix [101]. But since more than 70% of Indian population still resides in the rural areas, cancer of cervix still remain to be a leading cause of cancer related morbidity and mortality in Indian

women. According to hospital based cancer registries (HBCR), carcinoma of cervix is the most common cancer in women in Chennai and Bangalore[101].

Infection with high risk variants of HPV is considered to be the most important etiological factor for the development of carcinoma of cervix. Others include early age of first sexual activity, multiple sexual partners, low socioeconomic status, poor personal hygiene, smoking, immunosuppression, along with other environmental and genetic factors.

The various prognostic factors of carcinoma of cervix include age, stage, tumor size, depth of invasion, lymphovascular invasion, parametrial involvement, vaginal involvement and nodal status. In this study, the role of COX2 expression as a prognostic marker for carcinoma of cervix is being evaluated by comparing COX2 expression with other established poor prognostic factors.

Kosary et al. analysed relative survival rates in 17,119 cases of cervical cancer and showed that FIGO stage, histological grade, age at diagnosis and lymph node status are all independent prognostic factors[102].

Geetha et al. by a hospital based retrospective study[103] and **Viladiu et al**.[104]found that clinical stage is the only independent prognostic factor for carcinoma of cervix.

According to **Baalbergen et al.** FIGO stage, grade and lymph node metastasis are the most important prognostic factors[105].

COX2 (cyclooxygenase 2) is a key enzyme in prostaglandin metabolism. It is an inducible enzyme unlike COX1 which is constitutively present in all cells. COX2 enzyme has been studied extensively and its concentration has been found to be increased in many tumors including malignancies of oesophagus, colon, pancreas, cervix etc.

It is noted that COX2 expression is involved in both early and late stages of carcinogenesis [106]. Altered COX2 expression is noted in several malignanttumors because COX-2 is present independently in cells during the early stages of cell differentiation or replication[107, 108]. **Bandyopadhyay et al.**[109] has shown that COX2 is expressed more in invasive malignancies than in situ carcinoma of cervix. He also emphasised the need for further studies to determine the role of selective COX2 inhibitors in management of cervical carcinoma.

EPIDEMIOLOGY OF CERVICAL CARCINOMA IN OUR DEPARTMENT (JANUARY 2014 – DECEMBER 2014)

In the study period of 12 months from January 2014 to December 2014 a total of 511 cervix carcinoma cases were received in the Department of Pathology, Institute of Obstetrics and Gynaecology, Egmore, Chennai. Of these, 502 were cervix biopsies and 9 were hysterectomies. The statistics for the year 2014 are as follows:

- Among the 502 small biopsies, 466 were squamous cell carcinoma, 27 were adenocarcinoma, 5 were undifferentiated carcinoma, and one each of clear cell carcinoma of cervix, malignant mixed mulleriantumor of cervix, poorly differentiated carcinoma and adenosquamous carcinoma.
- Among the 9 hysterectomies, 3 were squamous cell carcinoma of moderately differentiated grade, 3 were squamous cell carcinoma - poorly differentiated grade and 3 were adenocarcinoma well differentiated grade.
- The most common type of carcinoma of cervix was squamous cell carcinoma (92.36%), followed by adenocarcinoma (5.87%)

THE CURRENT STUDY GROUP

This was a retrospective study involving 50 cases conducted at the Department of Pathology, Institute of Obstetrics and Gynaecology, Egmore, Chennai. Since only 9 cases of Werthims hysterectomy cases were received during this period that satisfied the inclusion criteria, all of these were included in our study. Remaining 41 cases were small biopsies which were selected randomly.

In our study group, the age of patients with carcinoma of cervix range from 40 – 80 years with mean age of 55.62 years. Maximum number of patients (36%) were of the age group 40-50years, closely followed by 51- 60 years age group (34%). No patients were present of age <35 years or >80 years. This is in accordance to multivariate analysis on Prognostic factors for

squamous cell cervical cancer by **Annika Lindström** were the mean age was 56.8years. Study by **Geetha et al.** also showed that occurrence of carcinoma of cervix is low in women of age <30 years and >70 years[103].

In our study group, carcinoma of cervix was found most commonly in post menopausal females (78%). The incidence in menstruating females were 22%.

Of the 50 cases included in our study, most common stage was stage IIIB which comprised 16 cases (32%) followed by stage IIB and stage IIIA each of which comprising 8 cases (16%) each.

The incidence of parametrial involvement in or study was 64%, vaginal involvement was 76% and clinically identifiable lymph node enlargement was 86%.

In the study group of 50 cases, the most common histological subtype of carcinoma was squamous cell carcinoma, followed by adenocarcinoma. This was similar to the study by **Kosary et al** and **Ferrandina et al**[110]

On comparing the age of the patients with COX2 expression, it was found that the expression of COX2 was lesser in younger females i.e 40-50 years age group (33.33%) when compared to females of older age group i.e age more than 50 years (40. 26%). So COX2 expression pattern in our study was correlating with age of the patient, which is an established poor prognostic factor. **Ferrandina et al** showed a higher incidence of COX2 positivity in older patients when compared to younger females. **Miaoling et al**[85] also

showed a similar incidence. But study by **Manchana et al** failed to demonstrate a significant correlation between age of the patient and COX2 expression[111].

On comparing menstrual status and stage of the disease, it was noted that there was a statistically significant correlation between these two with a p value of 0.004. This indicated that younger age group females presented in the early stages of disease when compared to older age group females.

When the size of the tumors were compared with COX2 expression, it was noted that smaller tumors showed less COX2 expression whereas it was significantly higher for larger tumors. This was found to be statistically significant with a p value of 0.007. Such a statistically significant correlation between tumor size and COX2 expression was also noted in studies by **Kim et al**[112] , **Miaoling et al** and **Ferrandina et al** But a statistically significant correlation was not observed in studies by **Khunamornpong et al**[113]and**Manchana et al.**

In our study, no correlation was noted on comparing COX2 expression with parametrial involvement. Positive association between COX2 expression and parametrial invasion has been noted in invasive cervical carcinoma stage IB by **Ryu et al**[80]**Manchana et al, Ferrandina et al** and **Miaoling et al**also demonstrated statistically significant correlation between COX2 expression and paramertial involvement by the tumor. No association with pararmetrial invasion was noted by **Kim et al**[114]. and **Khunamornpong et al.**

When COX2 expression was correlated with vaginal involvement by the tumor, it was noted that tumors with vaginal involvement were showing increased COX2 expression than those in which vagina was free of tumor. Vaginal involvement by the tumor has not been studied specifically in any of the studies.

In our study group, COX2 expression was found to be higher in patients with lymph node enlargement detected clinically. This was found to be statistically significant with a p value of 0.026. This is supported by studies by **Miaoling et alManchana et al, Khunamornpong et al**and**Ryu et al**who also demonstrated statistically significant correlation between these two in their studies. No association between COX2 expression and clinically enlarged lymph nodes were noted by **Ferrandina et al**and **Kim et al.**

Statistically significant correlation between presence of lymphovascular emboli and positive COX2 expression has been described in studies by **Bandyopadhyay et al,Ryu et al, Kim et al** and **Khunamornpong et al.** In our present study no such comparison could be done because 82% of cases in our study were cervix biopsies.

In the present study, COX2 expression was associated with higher clinical (FIGO) stage of the disease. But this association was not found to be statistically significant. Statistically significant association between COX2 expression and FIGO stage were noted in studies by **Miaoling et al**and**Ferrandina et al.** Other studies like those by **Khunamornpong et al** showed no correlation between these two factors.

When COX2 expression was correlated with the histopathological subtype of carcinoma, it was seen that higher COX2 expression was present in squamous cell carcinoma (42%), than in adenocarcinoma (25%). This is not in concordance with studies by **Ferrandina et al,Yong Bae Kim et al**[114]**Manchana et al**and **Bandyopadhyay et al** where higher COX2 expression was noted in adenocarcinoma than in squamous cell carcinoma.

On comparing COX2 expression with histological grade higher expression was seen in poorly differentiated SCC than in well and moderately differentiated grades. But this was not statistically significant. No such associations were noted in adenocarcinoma grades. This result is in concordance with studies by **Ferrandina et al, Dursun et al**[115]**, Lee JS et al**[116] and **Bandyopadhyay et al.**

According to our study, COX2 expression correlated significantly with size of the tumor, clinically detected enlarged lymph nodes and increased age of the patients, each of which are independent prognostic factors of carcinoma of cervix. Hence it can be concluded that positive COX2 expression can be considered as a poor prognostic marker and can aid in determining the treatment options for these patients.

SUMMARY

SUMMARY

- In the study period of 12 months from January 2014 to December 2014 a total of 511 cervix carcinoma cases were received in the Department of Pathology, Institute of Obstetrics and Gynaecology, Egmore, Chennai.
- Of these, hysterectomy cases were 9(1.76%) and small biopsies were 502 (98.24%).
- In the present study, the age of patients with carcinoma of cervix range from 40 – 80 years with mean age of 55.62 years. Maximum number of patients (36%) were of the age group 40-50years, closely followed by 51- 60 years age group (34%).
- In our study group, carcinoma of cervix was found most commonly in post menopausal females (78%). The incidence in menstruating females were 22%.
- Of the 50 cases included in our study, most common stage was stage IIIB which comprised 16 cases (32%) followed by stage IIB and stage IIIA each of which comprising 8 cases (16%) each.
- The size of tumor ranged from less than 0.5 cm to 8cm. Most of the tumors (80%) were more than 4 cm in size.
- 64% of tumors had parametrial involvement, 24% upto the lateral pelvic wall. 36% of cases had no parametrial involvement.
- Vaginal involvement was present in 76% of cases.
- 86% of the cases under study had enlarged lymph nodes which were detected radiologically, whereas in 14% of cases nodes were not detected.

- In our study group of 50 cases squamous cell carcinoma was the most common histological type. Among squamous cell carcinomas, most common was moderately differentiated grade (54%), followed by poorly differentiated grade (36%).
- When the menstrual status and stage of the disease were compared, it was noted that menstruating females presented in early stages of the disease, whereas postmenopausal females presented in advanced stages of disease. This correlation was statistically found to be significant with a p value of 0.004.
- When age of the patients was correlated with COX2 expression, it was found that the expression of COX2 was lesser in younger females i.e 40-50 years age group (33.33%) when compared to females of older age group i.e age more than 50 years (40. 26%).
- In our study, it was noted that smaller tumors showed less COX2 expression whereas it was significantly higher for larger tumors. This was found to be statistically significant with a p value of 0.007.
- When COX2 expression was compared with the parametrial involvement of the tumor no positive correlation was noted.
- When COX2 expression was correlated with vaginal involvement by the tumor, it was noted that tumors with vaginal involvement were showing increased COX2 expression than those in which vagina was free of tumor.
- On comparing the COX2 expression with nodal status of the patients, it was noted that among cases with enlarged nodes, 44.2% had positive COX2

expression. This was found to be statistically significant with a p value of 0.026.

- On comparing COX2 expression with clinical stage of the disease, it was noted that COX2 expression was more in tumors of higher clinical stage.
- In our study it was seen that higher COX2 expression was present in squamous cell carcinoma (42%), than in adenocarcinoma (25%).

CONCLUSION

CONCLUSION

- Cervical carcinoma ranks fourth among the leading causes of cancer worldwide. In India, it is the second leading cancer killer next only to carcinoma breast.
- Several studies, including our present study have pointed towards the possibility of considering COX2 as a poor prognostic marker for cervix carcinoma.
- Immunohistochemistry is an easier and effective method for identification of prognostic markers early in the course of disease so that patients can be categorised and appropriate treatment protocol decided.
- As surgical pathologists, our role does not end merely in diagnosing a malignancy or classifying it, but it is our responsibility to help the clinician in deciding the appropriate treatment for patients.
- In this era of targeted therapy, more studies should be conducted to detect possible theranostic markers so that targeted therapy can be implemented effectively against this common killer disease.

ANNEXURES

ANNEXURE I

INFORMED CONSENT FORM

Title of the study : **"EXPRESSION OF CYCLOXYGENASE-2 IN CERVICAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL VARIABLES".**

Name of the Participant:
Name of the Principal (Co-Investigator) :
Name of the Institution : Institute of Pathology, Madras Medical College.
Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in **"EXPRESSION OF CYCLOXYGENASE-2 IN CERVICAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL VARIABLES".**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the cervical biopsies and resected cervical specimens will be subjected to immunohistochemistry and histopathological examination.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understand that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:
Name and signature / thumb impression of the participant (or legal representative if participant incompetent)
Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு : **கர்பப்பைவாய் புற்றுநோயில் சிறப்பு குறியீடு (Cycloxygenase-2) தன்மையை கண்டறிந்து, நோய் குறியியல் காரணிகளுடன் ஒப்பிடுதல்.**

சென்னை மருத்துவக் கல்லூரி நோய்குறியியல் துறையில் பயிலும் முதுகலை மருத்துவர் திவ்யா .N, அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில் பங்குகொள்ள ..... ஆகிய நான் முழு மனதுடன் சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்ப்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

நான் கர்பப்பைவாய் புற்றுநோய் கட்டி நோய்கள் குறித்த இந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

நான் என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

எனக்கு அறுவை சிகிச்சை செய்யப்பட்டு நோய்க்குறியியல் துறையில் சதைப் பரிசோதனைக்கு பயன்பட்ட மெழுகுக்கட்டிகளை வைத்து ஆராய்ச்சி மற்றும் சிறப்புப் பரிசோதனை செய்யது கொள்ள சம்மதம் தெரிவிக்கிறேன்.

பங்கேற்பாளர் கையொப்பம்..... இடம் :..... தேதி :.....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் .....

ஆராய்ச்சியாளர் கையொப்பம்..... இடம் :..... தேதி :.....

ANNEXURE II

PROFORMA

Name	:	
Age	:	
IP Number	:	
Biopsy No	:	
Presenting symptoms	:	post coital bleeding/post-menopausal bleeding
Menstrual status	:	menstruating/ post-menopausal
Speculum Examination	:	growth present/ absent
Per Vaginal Examination	:	parametrial& vaginal invasion
Per Rectal Examnation	:	rectal invasion
Ultrasound findings	:	
CT findings	:	
Cystoscopy findings	:	bladder mucosal involvement
Clinical diagnosis	:	
Type of specimen	:	cervix biopsy/ hysterectomy
Gross	:	growth present/ absent
Microscopy	:	Histological diagnosis according to WHO classification
IHC (COX2 expression)	:	negative/ mild/ moderate/ strong positivity

ANNEXURE III

2009 modification of FIGO staging of carcinoma of cervix

Stage	Definition
I	Cervical carcinoma confined to uterus (extension to the corpus should be disregarded)
IA	Invasive carcinoma diagnosed only by microscopy; all macroscopically visible lesions, even with superficial invasion, are stage IB
IA1	Stromal invasion no greater than 3.0 mm in depth and 7.0 mm or less in horizontal spread
IA2	Stromal invasion more than 3.0 mm and not more than 5.0 mm with a horizontal spread of 7.0 mm orless
IB	Clinically visible lesion confined to the cervix or microscopic lesion greater than IA2
IB1	Clinically visible lesion 4.0 cm or less in greatest dimension
IB2	Clinically visible lesion more than 4.0 cm in greatest dimension
II	Tumor invades beyond the uterus but not to pelvic wall or to lower third of the vagina
IIA	Without parametrial invasion
IIA1	Clinically visible lesion 4.0 cm in greatest dimension
IIA2	Clinically visible lesion >4 cm in greatest dimension
IIB	With parametrial invasion
III	Tumor extends to the pelvic wall and/or involves lower third of vagina and/or causes hydronephrosis or nonfunctioning kidney
IIIA	Tumor involves lower third of vagina with no extension to pelvic wall
IIIB	Tumor extends to pelvic wall and/or causes hydronephrosis or non-functioning kidney
IV	The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum.
IVA	Spread of the growth to adjacent organs
IVB	Spread to distant organs

ANNEXURE IV

MODIFIED WHO CLASSIFICATION OF CERVIX CARCINOMA

- **Squamous cell carcinoma**
  - Microinvasive (early invasive) squamous cell carcinoma
  - Invasive squamous cell carcinoma
    - Keratinizing
    - Nonkeratinizing
    - Basaloid
    - Verrucous
    - Warty
    - Papillary
    - Squamotransitional
    - Lymphoepithelioma-like carcinoma
- **Adenocarcinoma**
  - Usual type adenocarcinoma
  - Mucinous adenocarcinoma
    - Endocervical type
    - Intestinal type
    - Signet-ring type
  - Minimal deviation
  - Villoglandular
  - Endometrioid adenocarcinoma
  - Clear cell adenocarcinoma
  - Serous adenocarcinoma
  - Mesonephric adenocarcinoma
- **Other epithelial tumors**
  - Adenosquamous carcinoma
    - Glassy cell variant
  - Adenoid cystic carcinoma
  - Adenoid basal carcinoma
  - Neuroendocrine tumors
    - Carcinoid
    - Atypical carcinoid
    - Small cell carcinoma
    - Large cell neuroendocrine carcinoma
  - Undifferentiated carcinoma

ANNEXURE V

IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4µ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated at 58ºC for overnight.
3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.
5. The sections were washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.
7. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 to 25 minutes.
8. The slides were then cooled to room temperature and washed in running tap water for 5 minutes.
9. The slides were then rinsed in distilled water for 5 minutes.
10. Wash with appropriate wash buffer (phosphate buffer) for 5 minutes x 2 changes.
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides in phosphate buffer for 5 minutes x 2 changes.
13. Cover the sections with power block for 15 minutes.
14. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 45 minutes.
15. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
16. The slides were covered with Super Enhancer for 30 minutes.
17. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
18. The slides were covered with SS Label for 30 minutes.
19. Wash in phosphate buffer for 5 minutes x 2 changes.
20. DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1ml of DAB buffer.
21. DAB substrate solution was applied on the sections for 8 minutes.
22. Wash with phosphate buffer solution for 5 minutes x 2 changes.
23. The slides are washed well in running tap water for 5 minutes.
24. The sections were counterstained with Hematoxylin stain for 2 seconds (1 dip).
25. The slides are washed in running tap water for 3 minutes.
26. The slides are air dried, cleared with xylene and mounted with DPX.

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MASTER CHART

serial no.	biopsy no	age	menstrual status	stage	tumor size	parametrialinvolv	vagina involv	nodal status	type of specimen	HPE diagnosis	COX 2
1	3	50	PMP	4	>4	2	2	P	CERVIX BIOPSY	2	1+
2	36	60	PMP	7	>4	3	3	P	CERVIX BIOPSY	6	1+
3	68	50	PMP	6	>4	2	2	P	CERVIX BIOPSY	2	N
4	156	65	PMP	3	0.5 - 4	1	2	P	CERVIX BIOPSY	5	N
5	189	60	PMP	6	>4	3	3	P	CERVIX BIOPSY	4	3+
6	198	42	Menstruating	5	>4	1	1	P	CERVIX BIOPSY	2	3+
7	269	55	PMP	6	>4	3	2	P	CERVIX BIOPSY	3	2+
8	272	65	PMP	4	>4	2	2	P	CERVIX BIOPSY	3	1+
9	431	57	PMP	6	>4	2	2	P	CERVIX BIOPSY	2	N
10	454	55	PMP	7	>4	2	2	P	Hysterectomy	3	N
11	461	40	Menstruating	6	>4	2	2	P	CERVIX BIOPSY	2	2+
12	505	50	PMP	5	>4	1	3	P	CERVIX BIOPSY	3	1+
13	538	48	PMP	6	>4	3	3	P	CERVIX BIOPSY	2	N
14	582	65	PMP	5	>4	2	3	P	CERVIX BIOPSY	6	N
15	601	52	PMP	4	>4	2	2	P	CERVIX BIOPSY	3	N
16	602	75	PMP	3	>4	1	2	P	CERVIX BIOPSY	3	2+
17	644	48	Menstruating	6	>4	2	3	P	CERVIX BIOPSY	2	2+
18	675	65	PMP	6	>4	3	3	P	CERVIX BIOPSY	2	N
19	848	42	Menstruating	4	>4	2	1	A	CERVIX BIOPSY	2	1+
20	851	65	PMP	4	>4	2	1	P	CERVIX BIOPSY	2	1+
21	939	55	PMP	6	>4	2	2	P	CERVIX BIOPSY	2	2+
22	995	58	PMP	3	0.5 - 4	1	2	P	CERVIX BIOPSY	1	1+
23	1009	62	PMP	4	>4	2	2	P	CERVIX BIOPSY	7	1+
24	1041	55	PMP	6	>4	3	3	P	CERVIX BIOPSY	2	N

25	1072	60	PMP	5	>4	3	3	P	CERVIX BIOPSY	1	2+
26	1179	50	PMP	5	>4	2	3	P	CERVIX BIOPSY	2	2+
27	1532	40	Menstruating	2	0.5 - 4	1	1	P	Hysterectomy	2	N
28	1598	40	Menstruating	1	<0.5	1	1	A	Hysterectomy	3	1+
29	1685	65	PMP	6	>4	3	2	P	CERVIX BIOPSY	1	1+
30	1841	60	PMP	3	>4	1	2	P	CERVIX BIOPSY	3	3+
31	1857	50	PMP	2	>4	1	1	A	Hysterectomy	4	N
32	1889	56	PMP	4	>4	1	2	P	Hysterectomy	3	2+
33	1952	53	PMP	6	>4	3	2	P	CERVIX BIOPSY	3	2+
34	1953	45	Menstruating	3	>4	1	2	P	CERVIX BIOPSY	2	1+
35	2021	41	Menstruating	2	0.5 - 4	1	1	A	CERVIX BIOPSY	2	N
36	2024	60	PMP	5	>4	2	2	P	CERVIX BIOPSY	2	1+
37	2034	65	PMP	5	>4	2	3	P	CERVIX BIOPSY	1	2+
38	2134	60	PMP	7	>4	2	3	P	CERVIX BIOPSY	2	1+
39	2135	57	PMP	2	0.5 - 4	1	1	P	CERVIX BIOPSY	2	N
40	2220	62	PMP	5	>4	2	3	P	CERVIX BIOPSY	3	3+
41	2242	67	PMP	6	>4	3	3	P	CERVIX BIOPSY	3	2+
42	2309	40	Menstruating	2	o.5 - 4	1	1	A	Hysterectomy	4	1+
43	2676	49	PMP	4	>4	2	1	P	CERVIX BIOPSY	9	2+
44	2743	65	PMP	6	>4	2	2	P	CERVIX BIOPSY	2	2+
45	2784	65	PMP	6	>4	3	2	P	CERVIX BIOPSY	8	1+
46	2874	80	PMP	3	0.5 - 4	1	2	P	Hysterectomy	2	N
47	2980	62	PMP	6	>4	3	2	P	CERVIX BIOPSY	3	2+
48	2987	45	Menstruating	3	>4	1	2	P	CERVIX BIOPSY	5	2+
49	3067	60	PMP	2	0.5 - 4	1	1	A	Hysterectomy	3	N
50	3105	45	Menstruating	2	0.5 - 4	1	1	A	Hysterectomy	4	N

KEY TO MASTER CHART

PMP	:	Post-menopausal
Stage		
1	:	IA
2	:	IB
3	:	IIA
4	:	IIIA
5	:	IIIB
6	:	IIIB
7	:	IV
Parametrial involvement		
1	:	Parametrium is free
2	:	Parametrialinvolved, not upto pelvic wall
3	:	Parametrialinvolved uptopelvic wall
Vaginal involvement		
1	:	Vagina is free
2	:	Upper 1/3rd of vagina is involved
3	:	Lower 2/3rd of vagina is involved
nodal status		
P	:	Present
A	:	Absent

HPE diagnosis

1	:	Well differentiated SCC
2	:	Moderately differentiated SCC
3	:	Poorly differentiated SCC
4	:	Well differentiated ADC
5	:	Moderately differentiated ADC
6	:	Poorly differentiated ADC
7	:	Adenosquamous carcinoma
8	:	Undifferentiated carcinoma
9	:	Clear cell carcinoma

COX2

N	:	Negative
1+	:	<10% tumor cells positive
2+	:	10-50% tumor cells positive
3+	:	>50% tumor cells positive